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A Second Series of Field Experiments in Lincolnshire on the Chemical Treatment of Soil infected with *Heterodera schachtii.*

By Richard H. Hurst, B.Sc., Ph.D., F.I.C..

(*Senior Research Assistant for Biochemistry, Institute of Agricultural Parasitology, St. Albans.*)

and Mary T. Franklin, B.Sc.

(*Attached to the Institute of Agricultural Parasitology, St. Albans, by the Agricultural Research Council.*)

Previous experiments on the application of ferric oxide or of calcium cyanamide to "potato-sick" land were carried out at the Kirton Agricultural Institute. The results were reported by Hurst & Franklin (1937), and indicated that ferric oxide had no effect either on the yield of potatoes or on the hatching of *H. schachtii* larvae, but that calcium cyanamide showed promise and was worthy of further trial. Experiments with this chemical were, by the kind permission of Mr. J. C. Wallace, Principal of the Kirton Agricultural Institute, and with the co-operation of Mr. J. Wood, A.R.C.S., continued for a second year.

The treatments in 1936 were, (A) control, (B) calcium cyanamide, 30 cwt. per acre, and (C) ferric oxide, 12 cwt. per acre. It was decided to grow early potatoes in 1937 on the A and B plots, without further treatment other than the customary artificials, and to carry out an experiment on the C plots in which cyanamide had been applied in the autumn previous to planting. It was thought highly improbable that the presence of ferric oxide on these plots would be a complicating factor, particularly as it would be distributed throughout the soil during the rototilling. It was necessary to await an opportunity when weather conditions would allow the efficient use of the rototiller, and this occurred on December 22nd, when calcium cyanamide was applied at the rate of 40 c.p.a. The soil-moisture was 23·6%, which is too high for the optimum lethal action of cyanamide.

The plots were prepared in April, 1937, and treated with the artificial mixture recommended by the Kirton Agricultural Institute, at the rate of 13½ c.p.a. Eclipse potatoes were planted, with 16 ins. between the setts, and 27 ins. between the rows. There was no retardation in growth

on the cyanamide plots and where cyanamide had been applied in the previous autumn there was conspicuous absence of weeds. The plants on these plots were very much superior to those on the controls or on the plots which had been treated with cyanamide in the previous spring.

The crop was lifted in August and the yields are shown in Fig. I and in Table I. It will be seen that the yield from the C plots was easily the highest, and that the yield from the previous cyanamide B plots was as poor as that from the controls.

Plots 1 to 9

A 146 (39)	C 244 (39)	B 128 (42)
C 263 (44)	B 52 (30)	A 23 (17)
B 51 (22)	A 55 (34)	C 246 (62)

Plots 10 to 18

C 221 (62)	A 104 (38)	B 80 (37)
B 44 (27)	C 245 (63)	A 137 (56)
A 51 (34)	B 80 (32)	C 163 (63)

FIG. I.

Yield in lbs. of " ware " and (in parentheses) " chats."

A=Control 1937, following control 1936.

B=Untreated 1937, following calcium cyanamide 1936.

C=Calcium cyanamide 1937, following ferric oxide 1936.

TABLE I.

		A	B	C	Standard Error
Total Yield	Lbs. per plot	122	104	286	19·4
	Tons per acre	3·4	2·9	8·0	0·54
" Ware " only	Lbs. per plot	86	73	230	16·1
	Tons per acre	2·4	2·0	6·4	0·45

The action of the 30 c.p.a. of cyanamide applied to the B plots before the 1936 crop, must be interpreted during that year as due partly to its properties as a nitrogenous fertiliser and partly to a strong retardation of hatching of larvae from *H. schachtii* cysts. It was thought that the

relatively small number of new cysts formed in 1936 might lead to an increased yield in 1937, but it is apparent that variations in actual numbers of cysts do not necessarily lead to varying degrees of " potato sickness." Moreover, it will be shown elsewhere that when calcium cyanamide is applied to infected soil in quantity insufficient to be lethal, the hatching power of the larvae may, following a temporary period of retardation, be much stimulated.

Pot experiments with soil from the control plots were carried out. The soil was air-dried and sieved and then mixed as thoroughly as possible with dressings of calcium cyanamide equivalent to 20, 30 and 40 c.p.a. One gram of cyanamide per 1,000 grams of soil was, for this soil, taken as equivalent to a field dressing of 20 c.p.a. mixed to a depth of 9 inches. Eclipse seed potatoes were kindly supplied by Mr. Wood, and were planted in a duplicate series of pots containing the above dressings, one month after the chemical had been mixed with the soil. At the end of the growing period the pots were " turned out " and the numbers of cysts on the outer roots were counted. The results are shown in Table II and these, in common with those of previous pot experiments

TABLE II.

Treatment	Average number of (newly formed) cysts on outer roots.
Control	596
Calcium cyanamide 20 c.p.a.	301
Calcium cyanamide 30 c.p.a.	2
Calcium cyanamide 40 c.p.a.	Nil.

on Bedfordshire soil, indicate that there is a critical concentration of cyanamide above which no hatching of larvae takes place. The previous experiments with Bedfordshire soil showed that above this concentration the chemical was lethal to the cyst contents, and had not merely exerted a strong temporary retarding action on the hatching of larvae. The critical concentration of cyanamide referred to would depend presumably on the type of soil and the conditions of the experiment.

It is clear, however, that if 30 c.p.a. of cyanamide is not entirely effective in preventing the formation of new cysts, even in pot

experiments with sieved soil which has been intimately mixed with the chemical by hand, a much larger dressing would be necessary in the field to ensure that it came into contact with the cysts in sufficient amount to be lethal to the contained eggs.

ACKNOWLEDGMENTS.

The authors wish to thank Mr. J. C. Wallace for allowing these experiments to be carried out on the land of the Kirton Agricultural Institute, and Mr. J. Wood, A.R.C.S., for his co-operation throughout the work.

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REFERENCE.

HURST, R. H. & FRANKLIN, M. T., 1937.—"Field Experiments in Lincolnshire on the Chemical Treatment of Soil infected with *Heterodera schachtii*." *J. Helminth.*, XV (1), 9–20. (*W.L.* 11224b.)

Journal of Helminthology, Vol. XVI, *No.* 1, *February*, 1938, *pp.* 5–16.

On the Occurrence of *Heterodera* Cysts in Various Soils and on the Roots of *Agrostis stolonifera* L.

By Mary T. Franklin, B.Sc.

(Attached to the Institute of Agricultural Parasitology, St. Albans, by the Agricultural Research Council.)

During the microscopical examination of the debris collected by flotation from soil infected with the potato eelworm *Heterodera schachtii* Schmidt, the presence of small numbers of cysts unlike those typical of the potato strain has frequently been observed. The distinguishing characters of these cysts are their dark colour and their lemon-like shape. Similar cysts were seen by chance during observations on "clean" soil which was being used as a control to pot experiments with eelworm-infected soil. This "clean" soil had been obtained from a heap of top-spit material which had been removed from a field on Winches Farm known to have been under grass for at least twenty years, and probably longer. Potatoes grown in this soil have never been found with cysts on the roots, and it did not seem possible for the soil to have become infected with eelworm cysts from any external source. Samples of soil were therefore taken from a position close to the spot from which the "clean" soil had been removed, and from neighbouring fields, all of which were under grass, and were carefully examined for cysts. In one sample no cysts were found, but nine others all contained cysts, the numbers varying from one or two to twenty or thirty in 100 grams of dried soil.

Many of the cysts found in this soil were more or less crushed and torn, and the original shape was difficult to determine, but some were little damaged, and amongst these two distinct types were evident. Some were definitely lemon-shaped, showing clearly, in addition to the neck, a posterior protuberance in the region of the vulva. Others had an even contour posteriorly, the only irregularity in the outline of the cyst being the neck. The majority of the cysts were found to be quite empty, but about 29% contained empty egg shells, and 2·7% unhatched eggs.

The roots of plants growing in the most heavily infected areas were examined for cysts, but without success. As this was during the first fortnight of June it was thought possible that the parasite might be present in the roots in the larval stages. Representative plants were therefore dug up and the roots stained with acid fuchsin in order to find out if any eelworms were present. On examination under the binocular microscope a few nematodes were found in the roots of an unidentified grass from one of the samples. The nematodes were mostly young *Heterodera* females which had become flask-shaped but were not protuding through the roots : one or two first-stage larvae, a few later larval stages and two or three nearly mature males were also found. In all about twenty *Heterodera* at different stages of development were observed.

Examination of samples of soil from other parts of Winches Farm was then undertaken. Most of the samples came from fields which were under permanent pasture, or had been broken up not many years previously, but three were taken from small coppices. In every case cysts were found. Soil samples obtained from two comparatively new gardens (probably less than ten years old), from a field some distance from the Farm and from a meadow five or six miles away all contained slight infections. In most cases there were only one or two cysts per 100 grams of dried soil, and of those in which the original shape could be seen, about half appeared to be lemon-shaped, and the rest rounded posteriorly.

In one of the samples from the Farm, which yielded 75·5 cysts per 100 grams of soil, it was noticed that many of the cysts were evidently newly formed, and appeared to be full of embryonated eggs. This sample had been taken from the corner of a meadow which was fairly free from weeds, and which had been under grass for at least 20 years. By this time (October) it was thought that it might be possible to find cysts on their host roots. Pieces of the turf were therefore removed and brought into the laboratory for examination. After a careful search had been made, cysts were found on the roots of a grass which, in the absence of flowers, was provisionally identified as *Agrostis stolonifera* L. About a dozen cysts were found on the roots of this grass, but no other infected plants were discovered. The cysts were generally attached to the roots very close to their point of origin, and in some cases it appeared at first sight as if they had been formed in the stem and not the root, but on closer observation it was seen that they were actually attached to

the base of the root. The reason that few cysts were found on the more distal parts of the roots may be that in separating the roots from the turf the cysts were rubbed off, since all those examined were mature and easily detached from the root on which they had developed.

Morphology of *Heterodera* Cysts from *Agrostis stolonifera*.

Although few cysts were found actually attached to roots of *A. stolonifera*, many others of a similar appearance to these were found closely associated with the roots and had evidently been formed upon them, since there were very few other roots present. The cysts are of a light brown colour, almost tan in many cases. When examined under the microscope in reflected light the eggs can often be seen inside. This seems to indicate that the cyst wall is slightly thinner than is usual in *H. schachtii*, where the eggs are not often visible through the wall. The general shape of the cyst is ovoid, and there is no prominence in the region of the vulva. It is sometimes possible to see the vulva fairly easily as a pore, occasionally surrounded by a darker coloured area, but it lies flush with the general outline of the cyst wall. The neck is very variable in length, and in many cases it is bent and may even be doubled back sharply along the cyst body. This may be due to the fact that the cysts are formed in a comparatively solid and unyielding medium, and are unable to expand in the normal manner with the neck straight out in a line with the long axis of the body, as is usual in those cysts formed on plants growing in cultivated land. On account of the frequency with which the necks were more or less bent, it was found impossible to measure them accurately. The average measurements of 100 cysts are given in Table I, the breadth being the greatest breadth, and the length being the greatest length excluding the neck.

That the cyst body is definitely more ovoid than that of the potato strain of *H. schachtii* is shown by the ratio of length to breadth, which averages 1·479 in the cysts found on *Agrostis*, whereas it is 1·128 in 20 cysts of the potato strain, which were measured excluding the necks. All the other known strains of *H. schachtii*, except the cysts occurring on the tomato, which are almost identical with those from potatoes, differ in being definitely lemon-shaped (see figs. 1–9). It will be seen from the figures given in Table II that the ratio of length to breadth for the cysts found on grass roots is on the average greater also than that for most lemon-shaped strains of *H. schachtii*.

TABLE I.

Dimensions of cysts from *Agrostis stolonifera*.
(Average of 100 measurements.)

Length in mm. (excluding neck)			Breadth in mm.			Length/Breadth		
Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
0·654	0·337	0·490	0·504	0·208	0·336	2·117	1·144	1·479

TABLE II.

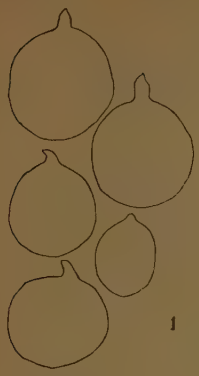
Length/Breadth for cysts of different strains of *H. schachtii*.
(Average for 20 cysts. All excluding neck.)

Host Plant	Maximum	Minimum	Average
Potato	1·333	1·000	1·128
Tomato	1·427	1·025	1·228
Marram	1·427	1·174	1·298
Pea	1·510	1·222	1·340
Oat	1·702	1·221	1·412
Mangold	1·690	1·249	1·544

An interesting feature of the cysts found on *A. stolonifera* is the presence on a few of them of a thin coating of a white material. This resembles a substance which can be observed occasionally on mature cysts of the oat strain of *H. schachtii* when these are newly formed and are still attached to the host root. A similar coating is present on recently matured cysts of the beet and mangold strain, but this soon disappears when the cysts drop off the host roots into the soil. When examining cysts found on marram grass the writer has observed also, in some cases, a crisp white coating which, under pressure, cracks and comes off the cyst cleanly. The coating found on some of the *Agrostis*-strain cysts is less hard, and if the cyst has been soaking in water it is quite soft, but it can be peeled

Heterodera cysts of different strains. (× about 25).

1. Potato strain. 2. Strain from *Agrostis stolonifera* L. 3. Strain from tomato. 4. Pea strain. 5. Lemon-shaped cysts from pasture soil. 6. Oat strain. 7. Beet strain. 8. Cysts from Leeds bowling green. 9. Cysts from marram grass (*Psamma arenaria* Beauv.).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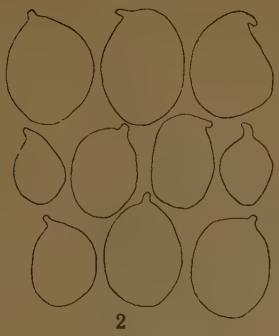
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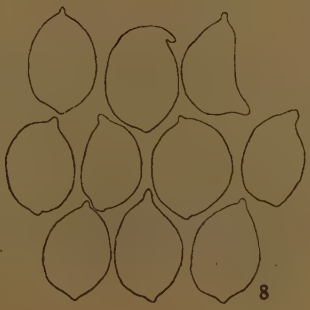
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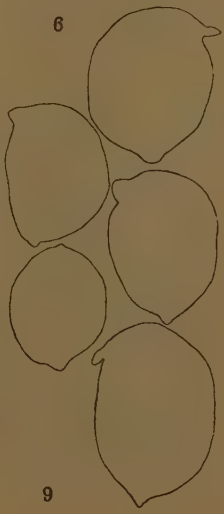
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off the cyst in the same manner. This may be compared with the sub-crystalline layer figured by Strubell (1888), but it differs in being complete and not fragmentary. It also resembles the white coating of crystalline substance described by Thorne (1928) covering the females of *Heterodera punctata*.

The cyst walls of ten cysts from *Agrostis* were examined in surface view at a magnification of about 1,100 diameters. In every case the walls had a speckled appearance which, on close examination, was seen to be due to minute dots arranged fairly regularly in rows. These dots remain visible when one focuses up and down, but change in appearance from bright spots to dark specks tending to join together in rows. It seems probable, since they are not visible at one level only, that the dots are small pits or punctations. Fig. 10 shows the manner in which the punctations are arranged. Besides the punctations, reticular markings

10

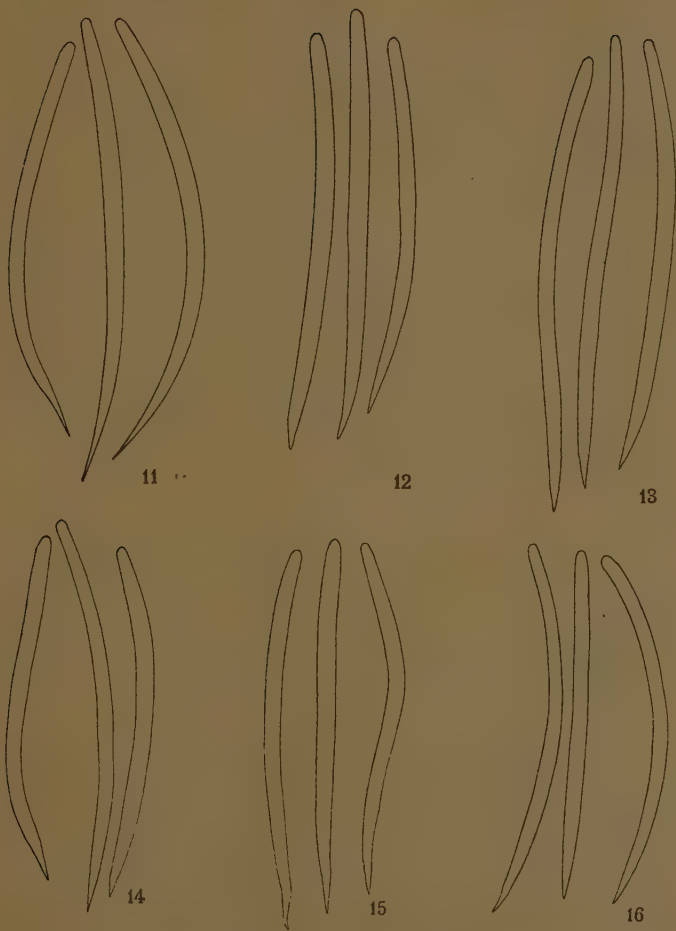
10. Arrangement of punctations on the wall of a cyst from *Agrostis stolonifera* L. (×about 1400.)

can often be seen on the cyst walls, but they are not constantly visible and appear to be irregular in form.

Ten cysts were dissected and the eggs were counted; the average number of eggs in the cysts was 125.

Ten eggs from each of ten cysts were measured and varied in length from 0·15 to 0·10 mm. with an average of 0·123 mm. In breadth they varied from 0·058 to 0·037 mm. with an average of 0·048 mm. The eggs therefore appear to be larger than those of the potato, oat and beet strains of *H. schachtii* which were measured by Triffitt (1929a), but are of similar dimensions to those of the mangold strain which she measured.

Larvae obtained by pressure from eggs from the grass-strain cysts were found to have an average length of 0·55 mm. This is greater than



Heterodera larvae. (× 108.)

11. From cysts parasitic on *Agrostis stolonifera* L. 12. *H. schachtii* potato strain. 13. *H. schachtii* oat strain. 14. From lemon-shaped cysts occurring in pasture soil. 15. *H. schachtii* pea strain. 16. *H. schachtii* beet strain.

that given by Thorne for *H. punctata* and by Triffitt for several strains of *H. schachtii*. The larvae of the mangold strain of *H. schachtii* are nearly as long with an average of 0·50 mm. The larval stylet measured 0.026 mm.; this is the same as that given by Triffitt for the length of stylet in the mangold strain.

The larvae obtained from cysts parasitic on *Agrostis* appear to taper more gradually posteriorly than those of other strains, the narrowing beginning at about one fifth of the body length from the tail, while in other strains it begins at about the level of the anus. (See figs. 11–16.) Measurements show that the tail is definitely more pointed than in the potato, oat, pea and beet strains of *H. schachtii*. This is of interest as some material consisting of stained grass roots containing nematodes, prepared by Dr. Goodey in 1932 and very kindly handed over to the writer, contains what appear to be larvae of *Heterodera* which have more finely pointed tails than is usual. These roots were obtained from a field where cysts have since been discovered, and it appears probable that the nematodes are of the same strain as those which have now been found attacking *Agrostis*.

Specific Identity of the Cysts parasitic upon *A. stolonifera*.

In many ways the cysts found parasitising *A. stolonifera* resemble those described by Thorne as *Heterodera punctata*. They are of similar dimensions and shape : the length is 0·49 mm. as compared with 0·52 mm. in *H. punctata*, but it is not known whether the latter measurement includes the neck or not. The average approximate length of the neck in 75 cysts from *A. stolonifera* was 0·0625 mm. : this would make the total length of the cyst about 0·5525 mm., which is slightly larger than the figure given by Thorne for *H. punctata*. The grass-strain cysts often possess a crystalline coating similar to that described for *H. punctata*. They also resemble the latter in parasitising a graminaceous host. The average egg content of 10 cysts found amongst *A. stolonifera* roots was 125 as compared with 78 for *H. punctata*, but this is a character which is liable to vary much in different populations of *H. schachtii* even when they are from the same host species. The cyst walls, when examined under a high power of the microscope appear to have punctations arranged in fairly regular rows. Thus it appears probable that the cysts found on *A. stolonifera* are similar to those described by Thorne for *H. punctata*.

The chief differences, according to Thorne, between *H. punctata* and *H. schachtii* are the rounded body terminus of the female of *H. punctata* as compared with the lemon-shape of *H. schachtii*, and the presence of punctations on the cyst wall of *H. punctata*, and not on that of *H. schachtii*. There are, however, strains of eelworm which have rounded cysts, and which are generally considered to belong to the species *H. schachtii*, notably the potato strain. Also, a preliminary examination of the cyst walls of potato, oat, pea and other strains of *H. schachtii*, shows what appear to be regularly arranged punctations. The size of the cyst and the egg content vary very much from cyst to cyst, and can hardly be considered to be sufficiently important characters to differentiate between species. The cysts of *H. punctata* thus do not appear to differ more from those of *H. schachtii* than cysts which have been looked upon as different strains of the single species *H. schachtii*, differ from one another. The larvae, however, from cysts parasitic upon *A. stolonifera* differ from those typical of *H. schachtii* in having more finely pointed tails.

Further investigations, now being undertaken, into the minute structure of the cyst walls of the different strains of *H. schachtii* may reveal differences sufficiently marked to be regarded as specific, or such similarity between *H. schachtii* and *H. punctata* as to remove the necessity for recognising two separate species.

For the present the cysts found on *A. stolonifera* are tentatively regarded as *H. punctata* Thorne.

The finding of this nematode parasitising a grass is interesting in view of Thorne's opinion that in Saskatchewan a grass was probably the original host of the strain attacking wheat.

Significance of the finding of *Heterodera* Cysts in Pasture Soils.

That the eelworm *Heterodera* is more generally present in soils than is usually supposed seems to be indicated by the finding of cysts in nearly all the samples of soil examined. It seems probable too that more than one strain, or possibly species, occurs, since two distinct types of cyst can be distinguished. Of the cysts recovered from the soil surrounding the roots of *Agrostis stolonifera* five per cent. were definitely lemon-shaped, and were obviously of a different strain from that found actually on the grass roots. It has yet to be discovered what is the host of the lemon-shaped cysts, and if larvae from the rounded cysts will attack other plants

besides *A. stolonifera*. It is possible that there is more than one strain of each type of cyst, and each strain may have a restricted or a wide host range.

Although *H. schachtii* has been recorded as a parasite of 30 different species of grasses (Corder, Buhrer & Thorne, 1936) few naturally occurring strains have been reported in Britain. Triffitt recorded *Heterodera* sp. on *Psamma arenaria* from Dawlish (1929b), and it was found on the same host by Dr. Triffitt and the writer at Southport, Lancashire. *H. schachtii* has been recorded by Triffitt on *Agropyrum repens* (1929a and b), *Poa annua* and *Lolium perenne* (1929a), and by Thompson (1935) on the grasses forming a bowling green at Leeds. In this case the turf was composed mainly of *Lolium perenne* and *Agrostis canina*. Cysts from the bowling green, which were handed to the writer by Dr. Triffitt, are small, dark coloured and definitely lemon-shaped. They resemble oat-strain cysts in shape and colour, but are smaller than is usual for this strain. Attempts to produce an infection on oats with these cysts have been unsuccessful. The cysts found on marram grass (*Psamma arenaria*) are also lemon-shaped, but are very much larger than most strains of *H. schachtii*, as can be seen in fig. 9. It is possible that some of the records of *H. schachtii* may refer to cysts of the *H. punctata* type, since the differences between the latter and the rounded strain of *H. schachtii* are slight. *H. punctata* may therefore occur on cultivated hosts in Britain although there has been no record of it.

It was suggested by Triffitt (1929b) that naturally occurring strains of *Heterodera*, such as that described by her on marram grass, may give rise to the forms which attack cultivated plants when these are grown frequently on land infected with indigenous strains of the eelworm. If *Heterodera* is really as widespread as the preliminary investigations now reported suggest, then it may be that the possible danger to cultivated crops is equally widespread. To what extent indigenous strains of eelworm are capable of attacking cultivated crops can only be discovered by observation and experiment. Thorne's description of the occurrence of *H. punctata* on wheat growing on land which had been under cultivation only for a short time suggests that his form at least can readily adapt itself to wheat, and other forms may be equally adaptable. The unaccountable outbreak of potato sickness caused by eelworm and described by Robertson (1937) in an area where the disease was practically unknown,

and under conditions unfavourable for its introduction suggests the possibility that under suitable conditions a native strain may quickly adapt itself to this host.

It is possible that the form of *Heterodera* known as *H. punctata*, having rounded cysts, has given rise to the rounded strains occurring in Britain on potatoes and tomatoes, and that the lemon-shaped form has given rise to the lemon-shaped strains of *H. schachtii* which attack oats, peas, mangolds, and other crops. The finding of ovoid cysts by Triffitt (1929b) on *Agropyrum repens* in an area in which potatoes were attacked and bore round cysts suggests that the rounded grass strain may have given rise to the form present on the potatoes, but it is uncertain whether this had occurred, or whether the infection on the grass had been produced by potato-strain eelworms, as was thought at the time. It is also possible that two distinct strains of the eelworm were present. A remarkable characteristic of the potato strain of *H. schachtii* in particular, is that once it has become specialised upon potatoes (supposing it has become adapted to them from some wild host), it is rarely found developing on other plants, at least under experimental conditions.

The occurrence of strains of *Heterodera* parasitic on the natural vegetation of a locality, and especially of strains resembling those which are capable of causing serious injury to cultivated plants, may give rise to confusion regarding the presence or absence of infection in a cultivated crop. Countries anxious to prevent the introduction of the potato eelworm prohibit the importation of seed potatoes grown in soil in which eelworm cysts have been found. If seed happens to have been grown in a locality where there is a strain of *Heterodera* parasitic upon some of the species of weeds commonly present, and especially if *H. punctata* occurs, with its cysts differing but slightly from the potato strain of *H. schachtii*, the crop cannot be certified as eelworm-free, even though the strain present may not attack the potato or any other cultivated crop. Thus it is important, both from the point of view of the possible spread of infection with seed potatoes, and of the potential danger to cultivated crops, that the host range of the naturally occurring strains of *Heterodera* should be known.

It is hoped that it may be possible to collect a sufficient number of cysts of the form found parasitising *A. stolonifera* to determine what plants this strain is capable of attacking. When more is known about

the host ranges of the various strains of *Heterodera* occurring naturally in the soil it will be possible to estimate with greater accuracy their significance in relation to cultivated crops.

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A Disease of the Australian Grass *Microlaena stipoides* R.Br. caused by a Nematode, *Anguillulina microlaenae* n. sp.

By Stella G. M. Fawcett, M.Sc.
(*Botany Department, The University of Melbourne.*)

Introduction.

There have been no previous records of nematode invasion of native grasses in Australia, although " Cockle " in wheat, caused by *Anguillulina tritici*, and root galls on wheat, due to *A. radicicola* are known to occur. Mr. J. E. Harrison, Senior Agrostologist, Department of Agriculture, Victoria, found *Microlaena stipoides* infected as herein described at Lorne in April, 1935. This seems to be the first record of the disease, but it has since been ascertained that it is widely distributed in Victoria.

Microlaena stipoides R. Br. is described by Ewart as " a slender perennial grass with a short rhizome and narrow leaves. Stems erect or nodding, 1 to 2 ft. high. Blades rather short, flat, acute, slightly hairy, or glabrous. Panicle narrow, 3 to 6 ins. long, with erect or pendant branches and pedicels." It favours a moist situation and grows in extensive patches.

As a result of infection by the nematode, galls are produced on the shoot structures of the plant (Fig. 16). They occur principally on the leaves, singly, or in clusters so large that the greater part of the surface of the leaf is hidden by them. In such extreme cases the leaves are twisted and deformed, but where there are only a few galls on a leaf no distortion occurs. Heavily infected shoots are dwarfed and unhealthy looking, but with a light infection no noticeable reduction in the size or vigour of the plant is apparent. Even when closely aggregated the outlines of the galls remain distinct and no compound galls have been observed ; but, some modification in shape is observed when the galls form a close mass. Galls are also commonly found on the leaf sheath, the stem, the axis of the inflorescence and upon the rhizome. They are occasionally found on stems which are enclosed by the leaf sheath ; in such a case the latter does not completely enclose the stem.

The inflorescence is not uncommonly attacked. Galls may be present on the glumes and often cause distortion of the grain. More uncommonly galls arise from invasion of the stamens or the ovary, when one or two

galls take the place of the normal grain. The formation of such galls leads to atrophy of the other flower elements if they are not also infected.

On making counts of the worms in each gall it is found that the numbers vary between one and twelve. Commonly there are four or five. The numbers of each sex in a gall are usually equal, with an occasional preponderance of one over the other. No increase or decrease in the complexity of the gall is found with the greater or smaller numbers of individual worms present. The difference is one of size.

Life History.

Towards the end of the summer the aerial parts of *Microlaena* and any galls present on them become dry. Adult worms contained in the galls die and the infective larvae become desiccated. By this time all the eggs have not hatched but these seem to be able to resist a certain amount of desiccation and often proceed with their development when moistened. With the onset of damp conditions, the basal parts of the galls decay rapidly, as less thickening occurs here than on top of the gall. The larvae revive and become very active. They are now at the first stage and do not change to the second stage until they enter young tissues to set up new galls. They are very active and make their way to the growing points of the seedlings and young shoots. It has been shown that seedlings are not susceptible to attack before the breaking of the coleoptile. It is also impossible to infect mature tissues. It is necessary that the worms reach the growing point to set up an infection and this condition (in some measure) protects the plant from infection once the culm has elongated. However, *Microlaena* has not a strong upright habit and the long stems frequently trail on the ground, leaving the growing point sufficiently near the ground not to be out of reach of the infective larvae.

On entering the tissues the worms change to the second stage. Three more moults take them to the adult stage. Reproduction is completely sexual, no parthenogenesis has been observed. As many as 2,000 eggs have been counted in a single gall where there were many worms of both sexes present. When one of each sex is present there are between 150 and 400 eggs laid. The eggs are deposited in an unsegmented condition and slowly become embryonated. Some distribution in time is effected by different rates of hatching of the eggs. Some hatch quickly—others slowly—so that new worms are appearing over quite a long period.

The adult worms survive for as long as conditions remain favourable after the eggs are laid. When dry conditions set in they die quickly.

There are several species of fungi which can grow through the gall tissue and infect the worms, completely filling the cuticle with a densely packed mass of hyphae. Eggs and larvae are attacked in the same way and are frequently bound together by the hyphae. These parasitic fungi are chiefly found in galls on the rhizomes and on stems near the ground. So far they have not been identified but there are two Phycomycetes concerned as well as one other. They will not grow on any of the culture media tried so far but only a small amount of infected material has been handled.

Morphology.

Female ... ... Length, 1·3–3 mm. commonly about 2 mm.
Breadth, 0·08–0·2 mm. commonly 0·14 mm.
Oseophagus, 0·10–0·19 mm.
Excretory pore, 0·10–0·21 mm.
Tail, 0·07–0·12 mm.
Stylet, 8–9μ.
α=18·8–12·2 β=18·7–9·3 γ=32–15 Vulva=87–88%

Male ... ... Length, 1·1–2·0 mm. commonly 1·3–1·5 mm.
Breadth, 0·055–0·115 mm. commonly 0·07–0·08 mm.
Oesophagus, 0·12–0·22 mm.
Excretory pore, 0·115–0·21 mm.
Tail, 0·055–0·11 mm.
Stylet, 8–9μ.
Spicules, 27–35μ.
Gubernaculum, 9–10.
α=22·5–17. β=13·9–8·3 γ=25·7–15·8

Adults.—The adults of both sexes rather closely resemble those of *Anguillulina tritici* and *A. agrostis* in general shape. The body gradually tapers anteriorly in both sexes. In the male the posterior region also tapers very gradually but in the female this is not so marked, the body decreasing rapidly in width between the vulva and the tail. In both sexes the tail is offset by a peg-like process. The head is flattened and button-like and narrower than the end of the body, from which it is marked off by a slight constriction. The lips are equal and fused, but distinct. The openings of the amphids are associated with the lateral lips as small pores. The amphids are fusiform and lie one on either side of the stylet.

The latter has the typical *Anguillulina* structure and is made up of a sharply conical anterior portion and a cylindrical posterior part which bears three prominent and equidistant knobs at the base. The muscular bands responsible for moving the stylet are attached to its base and are inserted at the junction of the head and body. The pharynx is cylindrical and, with the head, is lightly cuticularised. The oesophagus is continued backwards from the base of the stylet and in its anterior portion varies in width between $\frac{1}{4}$ and $\frac{1}{3}$ the corresponding width of the body. It gradually widens to form an indistinct median bulb in the centre of which the lining bears three small, often scarcely visible, crescentic thickenings. Posterior to the median bulb, the oesophagus gradually narrows to form the neck region which is often folded upon itself because of the forward thrust of the enlarging ovary and testis in the adults. The neck is encircled by the nerve ring and expands to form the posterior glandular part of the oesophagus. This is spatulate or roughly triangular in outline and its hinder portion lies over the top of the intestine. The substance of the glandular tissue is made up of three uninucleate cells—two small anterior ones and a large posterior one. The nuclei of all three may be easily distinguished in larvae and young adults but the two smaller nuclei are very inconspicuous in gravid worms. The dorsal gland is continued forward on the wall of the oesophagus, to open into the lumen by a duct immediately behind the stylet. The two subventral glands discharge just behind the crescentic thickenings of the median bulb (Fig. 1).

The walls of the intestine are composed of polygonal cells which present a tesselated appearance. They are well stocked with fat globules which make the whole very opaque. A short rectum leads to the anus.

The excretory pore occurs on the ventral surface near the opening of the oesophagus into the intestine. The cuticle bears fine striations 1μ distant from one another. They are interrupted by the lateral fields.

On teasing out the worms from green galls, it is found that the females are coiled watch-spring-wise with the ventral surface innermost (Fig. 4). They characteristically adopt this position when killed by heat or fixing. The vulva occurs far back on the body and has prominent rounded lips. It opens directly into the uterine cavity which is continued backwards as a post-vulval sac. Anteriorly it is somewhat dilated, forming a more or less distinct vagina. It then continues forward along the ventral

Anguillulina microlaenae n. sp.

Fig. 1.—Head of adult worm. Fig. 2.—Fourth stage larva (male) showing developing spicules. Fig. 3.—Fourth stage larva (female). Fig. 4.—Adult female.

surface as the uterus. The walls of this organ are cellular and thick, and one to three eggs are found in it at the one time. After travelling forward along the ventral surface for a considerable distance the uterus crosses to the dorsal side and expands to form a receptaculum seminis. Following this, a sphincter composed of a few large cells leads to the ovary which is continued forward into the body, gradually diminishing in width. Its termination is usually found in the oesophageal region, but it may be reflexed along the ventral surface. The cells of the ovary are polygonal and are arranged in two or three alternating series in its lower parts. Towards the termination the cells are, however, often uniseriate.

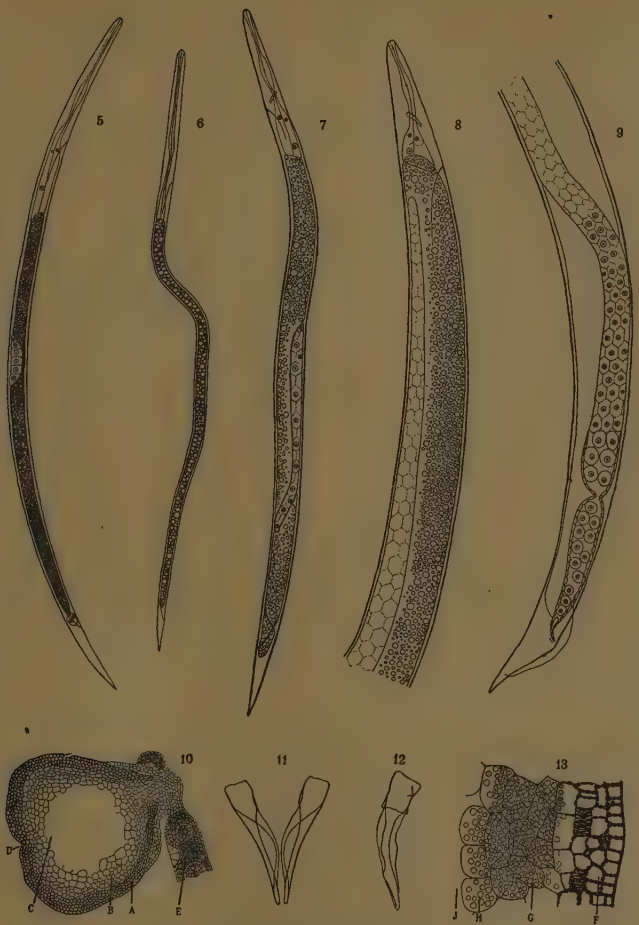
The male worms are smaller and slenderer than the females, and on being removed from the galls are considerably more active. On fixing, they too adopt a characteristic position, becoming bent to form almost a semicircle with the ventral surface outermost. The gonad is single and extends forward into the body almost to the oesophageal region, occasionally being reflexed from the middle region. The posterior part forms a well-marked vas deferens with thick cellular walls. At its junction with the testis there is a prominent sphincter. The cells of the testis are polygonal in outline and two or three of them take up the width of the organ. The gonad, in its posterior half travels along the ventral surface, and then turns abruptly across to the dorsal side and proceeds forward into the body. It is very seldom reflexed at its anterior termination but is frequently bent back upon itself from the point where it crosses the body.

The spicules are paired and divergent anteriorly. Each consists of a solid arcuate central portion which has a thin expansion arising on either side of it. These wings originate a short distance from the upper end of the spicule and are continued almost to the point. The inner expansion is bent over the central portion so that its edge almost approximates to that of the outer wing (Figs. 11, 12).

The gubernaculum is simple. The caudal alae arise somewhat in advance of the head of the spicules and are inserted near the tip of the tail. There are no papillae and the margins are faintly crenate because of the striations in the cuticle.

Eggs.—85–120μ long, 37–52μ wide, cylindrical with rounded ends, often slightly curved.

Larvae.—The first stage larvae are 0·5–1 mm. long and 0·009–0·012 mm. wide. They are very active and able to withstand desiccation. The



Anguillulina microlaenae n. sp.

Fig. 5.—Second stage larva. Fig. 6.—First stage larva. Fig. 7.—Third stage larva showing gonad now crossing to the ventral surface. Figs. 8 & 9.—Adult male. Fig. 10.—Section of mature gall. (A) sclerenchyma, (B) nutritive zones, (C) cavity in which the worms live, (D) funnel-shaped depression on upper surface, (E) normal leaf blade. Fig. 11.—Spicules; face view. Fig. 12.—Spicules; side view. Fig. 13.—Section of wall of mature gall. (F) outer sclerenchyma zone with simple pitted thickening, (G) inner nutritive zone—the cells of which contain starch grains, (H) the cells of the innermost layers are partly free, (J) cavity.

oesophagus is comparatively long and the genital primordium is seen as a small group of cells about midway down the intestine on the ventral side (Fig. 6). After entering the tissues of the host the larvae undergo a moult and change to the second stage, becoming much wider but not much longer. The genital primordium migrates to the dorsal side and increases in length. Meanwhile a small group of cells develops anterior to the anus but in contact with it (Fig. 5). A moult leads to the third stage. Here the worms begin to show their characteristic curvature, and a further increase in length and width is apparent. The gonad crosses from the dorsal to the ventral surface along which it grows and joins the group of cells at the anus (Fig. 7). A moult brings the larvae to the fourth stage in which the gonad increases in size and becomes differentiated. By the end of this stage the male accessory structures—spicules, gubernaculum and alae are complete, and the testis and vas deferens are distinct from one another. In the female differentiation of the gonad is complete, but the vulva is closed by the fourth stage cuticle.

After the final moult the vulva is open to the exterior and the caudal alae of the male are expanded. The gonads increase greatly in size and this accounts in some measure for the increase in size shown by the young adults. However, some increase in length also takes place. Previous to this increase in the adult stage the worms grow most rapidly in the third and fourth stages.

The small group of cells which develops near the anus in the second and third stage larvae is associated in the male with the formation of the spicules and gubernaculum. In the female it seems that it gives rise to the cells immediately surrounding the vulva but it is difficult to distinguish these accessory cells from those which are formed from the gonad.

Nomenclature.

From the foregoing description it is apparent that the nematode attacking *Microlaena* belongs to the genus *Anguillulina*. The adults of both sexes are elongate and worm-like, the oesophageal characters are typical—three uninucleate glands being present, the dorsal gland opening by a duct just behind the base of the stylet, the sub-ventral glands opening behind the crescentic thickenings of the median bulb. The male and female characters are also typical. In the female, the vulva is post-equatorial, the gonad is single and outstretched anteriorly and

has a post-vulval uterine sac. In the male, a gubernaculum and caudal alae are present. The spicules are paired and divergent anteriorly and taper towards the points.

Table to show the points of difference of *Anguillulina microlaenae* from related species.

Species of A.	*Oesophagus*	*Relation of oes. to intestine*	*Female Position*	*Ovary*	*Male Position*
A. tritici ...	median bulb distinct thickenings present	in contact but not overlapping	coiled	reflexed once or twice, cells in at least 3 series	ventral surface outermost
A. agrostis ...	,,	,,	coiled	reflexed once or twice, cells in a single series	dorsal surface outermost
A. graminis ...	,,	,,	slightly curved	reflexed cells in two or three series	ventral surface outermost
A. cecidoplastes	no median bulb or thickenings	,,	coiled	reflexed	indefinite
A. microlaenae	median bulb not distinct, thickenings present	oesophagus overlies intestine in posterior part	coiled	seldom reflexed cells in two or three series	ventral surface outermost

The adults of *Anguillulina microlaenae* strongly resemble those of *A. tritici*, *A. agrostis*, *A. graminis* and *A. cecidoplastes*.

It differs from the first three in the oesophageal characters, having no definitely marked off median bulb, although the crescentic thickenings are present. In addition, the glandular portion overlies the intestine. This is not seen in the three species mentioned. *A. cecidoplastes* is distinct because it lacks the crescentic thickenings in the median bulb.

The female of the species closely resembles those of the four similar species in the characteristic position it adopts when killed by heat or fixing. *A. graminis* can be partly eliminated here since it is only slightly curved under these conditions. In addition, the anterior end of the ovary in these four species is typically reflexed whereas in the nematode under consideration the ovary but seldom behaves in this manner.

The same curvature of the male worm with the ventral surface outermost is seen in *A. tritici* and *A. graminis*. The males of *A. agrostis* curve in the opposite direction—no details are available concerning the behaviour of *A. cecidoplastes* in this respect, but from Goodey's drawings it appears that the males assume an indefinite position.

The spicules are quite distinct from the four comparable species. In none of these is an inner enlargement reflected back so that it overlies an outer one.

From the above it is apparent that there are sufficient differences in morphology to regard the nematode attacking *Microlaena* as a distinct species. No attempt has been made to infect other grasses with the *Microlaena* nematode but under natural conditions other grasses growing near diseased *Microlaena* plants are not affected.

Structure of Normal Leaf Blade of *Microlaena*.

The leaf blades of *Microlaena* are from one to 6 ins. long and from $\frac{1}{8}$–$\frac{1}{4}$ in. broad. Denticules are present along the margins and on the cells immediately above and below the vascular bundles. Epidermal hairs may be developed in great numbers on both upper and lower surfaces but may be almost completely absent. A transverse section of unaffected leaf blade shows that it is made of an upper and lower epidermis with intervening mesophyll and vascular bundles.

The upper epidermis consists of flat tubular cells with uneven margins which lie in lines about six cells wide directly over the vascular bundles. At the edges of these lines of cells the stomata occur in one, or two alternating, series. Between these lines of cells, and thus alternating with the vascular bundles, are rows of bulliform cells. These are thin walled and larger than the ordinary epidermal cells. The cells of the lower epidermis are more regular in shape and size than those of the upper epidermis and are in addition slightly smaller. Immediately below the vascular bundles they often become thickened on all walls, and where the underlying cells are thickened as well, a projection on the surface is formed. Stomata are present in lines which correspond to those on the upper surface. There is a definite midrib in the lower part of the leaf but this becomes indistinct towards the tip. Two types of vascular bundle, large and small, may be distinguished. The midrib is similar in structure to the larger vascular bundles but is more prominent because

of the large amount of sclerenchyma developed around it, particularly towards the lower surface. On either side of the midrib there are one or two large vascular bundles. In between these larger bundles and between the outside ones and the edge of the leaf are the smaller bundles. Two or three of these occur between each of the large bundles and there are one or two of them between the edge of the leaf and the first large bundle.

The arrangement of the vascular elements in both types of bundles is similar and typical. In the large bundles the bundle sheath is very much thickened and when young is completely surrounded on the outside by a ring of large, non-chlorophyllous, parenchyma cells. On the top of the bundles this parenchyma sheath joins a patch of similar cells which reaches to the upper epidermis. Below the large bundles a similar patch of parenchyma occurs. Both of these parenchymatous areas are seen to be about five cells wide in transverse section. The lower patch soon becomes sclerenchymatous and often projects a considerable distance from the surface when the epidermal cells also become thickened.

The parenchyma cells above the vascular bundles do not become thickened so early, but in an old leaf some thickening can be observed. The cells of the upper epidermis, however, do not become sclerenchymatous.

The small vascular bundles are also surrounded by a ring of parenchyma cells which join similar areas above and below the bundles. No extensive areas of sclerenchyma are developed in connection with these cells, usually a small isolated group of three or four cells, both above and below the bundle, shows thickening. The mesophyll is devoid of conspicuous air spaces and occupies the intervals between the vascular bundles and reaches to the upper and lower epidermis.

The Galls.

Mature galls are small, approximately circular structures, from 1–3 mm. in diameter and 0·5–1·5 mm. high. When examined under low magnification the convex outer walls of the surface cells give the galls a rugose appearance. The main projection is from the upper surface of the leaf, only a small boss of gall tissue being visible from below. The base of the gall is situated in the mesophyll, *i.e.*, between the vascular bundles, which are pushed apart in the presence of a gall, but return to their normal course immediately above it. The galls are shortly turbinate in shape, the upper surface being flat. In the centre of the top of a gall

there is usually a small funnel-shaped depression which may represent the original point of entry of the worms (Fig. 10).

A series of vertical sections through young galls shows that the first signs of swelling are to be found in the mesophyll and epidermal cells. Vascular tissues lying near the point of attack become slightly enlarged and their elements may become scattered.

Depending on the extent of the affected tissue, all or part of the parenchyma elements associated with the vascular bundles in the immediate vicinity of the gall becomes indistinguishable from the swollen mesophyll cells. Thus, in a light infection, when the swelling affects only one side of each of two vascular bundles the sclerenchyma and parenchyma sheaths and the parenchyma of the bundles themselves are absent from those sides. When points of infection lie on either side of a bundle the sheaths and the parenchyma quite disappear. In both cases the vascular elements are distorted and displaced and although they show some enlargement and multiplication, these are not so great as in the mesophyll.

In a very young gall the worms can be seen lying in a cavity among the swollen mesophyll cells. The epidermal cells are stretched and enlarged to accommodate the swelling. Above the worms the cells are dead, and form a brown spot on the upper surface of the gall (Fig. 15). A certain amount of viscid sap is present among the cell remains and in the cavity (Fig. 15). Part of this at least is derived from the lysis of the cells, as it can be shown that cellulose is present in it. However, although there are obvious changes in the middle lamellae of the cells immediately surrounding the worms, no pectic substances can be detected in the sap. Some of the cells surrounding the cavity are incomplete, the others are rounded and swollen, and partly free from one another. The nuclei are greatly enlarged. Starch is present in the cells at an early stage, and soon, signs of thickening can be observed in the outer cells of the upper layers.

The young galls have a translucent green appearance and are lens-shaped in the early stages. As the galls develop they increase in size upwards and rapidly become opaque and whitish. At the middle stage of their development they are almost hemispherical. Then the cells of the upper periphery multiply rapidly and give the gall its flat top as the growth is in a centrifugal direction.

The continued growth of the cells surrounding the original point of entry of the worms, and therefore on top of the gall, is impossible, as they are by this time heavily thickened. At this stage the outline of the gall viewed from above is seen to be lobed and irregular although still roughly circular. When this proliferation is complete the gall has a smooth outline and has reached its final shape. The colour at this stage is brown.

On opening such a mature gall, the worms are found to be lying in a central cavity which, in addition, contains air and a small amount of viscid sap. A vertical section shows that the walls of the gall are 8–16 cells thick and are differentiated into an outer and inner layer.

The inner layer completely surrounds the cavity of the gall and is composed of large thin-walled cells. The innermost ones of these are empty and their walls are incomplete, possibly because of the lytic action of the secretions of the parasite. The rest are closely packed with small starch grains. The walls of these cells give the characteristic cellulose reaction with chlor-zinc-iodine.

The cells of the outer layer give no reaction with chlor-zinc-iodine, they are smaller and are seen to have greatly thickened walls. The thickening is of the simple pitted type and tests show that lignin is present. The greatest amount of thickening occurs in the cells of the upper surface of the gall, those towards the base showing progressively lighter thickenings. This is especially the case in the cells of the exterior of the base. But the more deeply seated cells of this basal region of the sclerenchyma zone show a greater amount of thickening than those on the exterior at this point. The cells of the sclerenchymatous layer usually contain no starch, the innermost ones may contain a few grains (Fig. 13).

Chloroplastids are absent from mature galls but a few are present in the middle stages of development when the galls are greenish white. Occasionally a mature gall is very deep purple in colour. This is due to the presence of an anthocyanin pigment which occurs in the sclerenchyma tissue. Most galls, however, are greenish or brown but may show a few pigmented cells on the sides. A deeply coloured gall may occur in the centre of a cluster of normal whitish galls or may occur singly.

It has been shown that *A. microlaenae* can only attack very young, and therefore, not heavily cuticularised cells. Thus the young epidermal cells would be permeable to the secretions and it would not be

necessary for the worms to enter through the stomata. The patch of dead cells on the upper surface of each young gall tends to support this idea, particularly as the centre of the dead area does not always coincide with one or other of the lines of stomata. Thus, the probable action of the worms on the tissues is as follows. The secretion in high concentration kills the surface cells, but the underlying cells are stimulated by the secretion at its optimum concentration. The worms make their way through the dead cells into the loosened mesophyll cells. Here the secretion has an inhibitory and lytic action on the cells immediately surrounding the worms, and a cavity is formed. The secretion diffusing into the deeper layers of cells—being weaker—has a stimulatory effect and the gall enlarges. Further away from the centre of infection the secretion is of such low concentration that the plant is able to neutralize it.

The structure of a mature gall on *Microlaena* resembles that of the gall produced on *Andropogon pertusus* by *Anguillulina cecidoplastes*. But, the *Andropogon* gall has three distinct layers, an inner nutritive zone, a median sclerenchyma and an outer parenchyma layer. The galls formed on *Microlaena* have only the two inner of the three zones. In addition, a purple anthocyanin pigment is invariably present in the outer layers of the galls on *Andropogon* but this is not frequently met with in *Microlaena* galls. Also, the main projection of galls on *Andropogon* is to the under surface of the leaf, whereas on *Microlaena* the galls project mainly from the upper surface.

Goodey draws attention to the resemblance between this complicated type of Nematode gall and Cynipid galls, in which the cavity where the parasites live is surrounded by a nutritive zone which is enclosed by a layer of cells with reticulate thickenings.

Goodey, following Kostoff and Kendall, regards the formation of a gall neither as a result of mechanical stimulation due to the presence of the parasite nor as a storehouse of food for it, but as " a reaction product on the part of the plant in its efforts to counteract the injury and control with antibodies the foreign substances that may be introduced by the gall former, or that may result from the disintegration of the contents of cells destroyed by the parasitic injury, the gall growth ceasing when the stimulative effects of these foreign substances are controlled and neutralized by antibodies produced by the plant " (Kostoff and Kendall).

The early stages of gall formation may be regarded as due to the unchecked action of the irritant substances upon the tissues of the host. Later the protective substances elaborated by the plant are produced in sufficient amount to neutralize the irritants, and the growth of the gall ceases. This neutralization of the effect of the secretions is expressed in the thickened walls of the sclerenchyma zone, and possibly by the production of the anthocyanin pigment.

It may also happen that, as the worms develop in the tissues, they lose the power of producing the irritant substances. When one considers that worms other than the infective stage are unable to set up a new infection this is not impossible. This suggested diminution of the toxic secretion may also be a factor in limiting the size of the gall.

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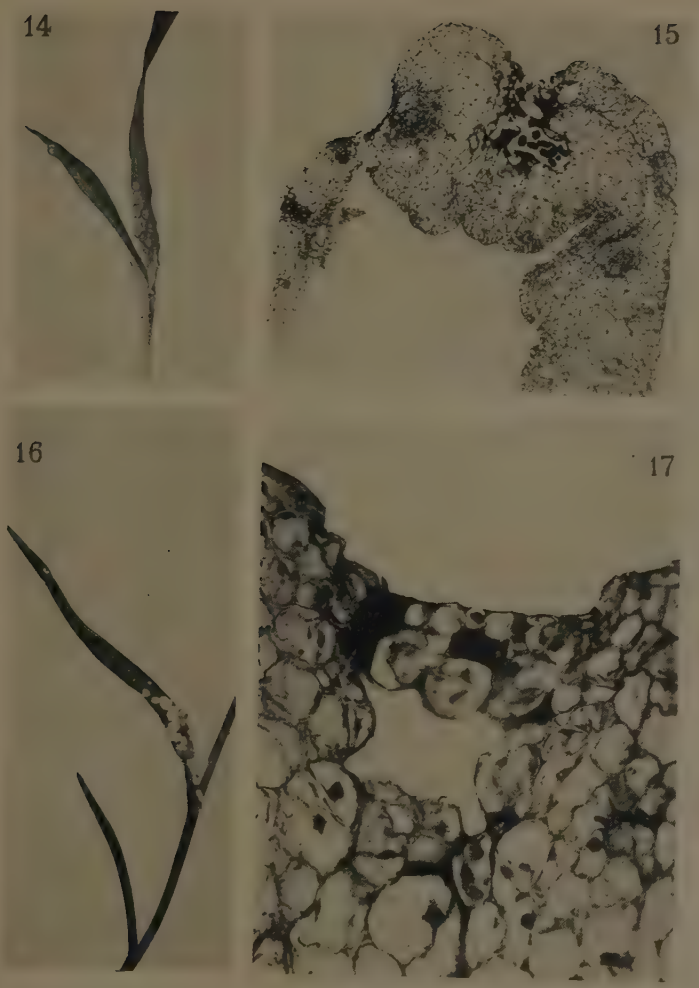

Natural infection of *Microlaena stipoides* with *Anguillulina microlaenae* n. sp.

Fig. 14.—Very young galls. Fig. 15.—Section of young gall showing dead cells on the upper surface and viscid sap in the cavity. Fig. 16.—Mature galls. Fig. 17.—Shows point of entry of the infective larvae. The dark material represents viscid sap resulting from lysis of cells. The swollen mesophyll cells, which are partly free from one another, have large nuclei.

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Field Experiments in Bedfordshire on the Chemical Treatment of Soil infected with the Potato Eelworm *Heterodera schachtii*, during 1936–37.

By Richard H. Hurst, B.Sc., Ph.D., F.I.C.

(*Senior Research Assistant for Biochemistry, Institute of Agricultural Parasitology, St. Albans.*)

and Mary T. Franklin, B.Sc.

(*Attached to the Institute of Agricultural Parasitology, St. Albans, by the Agricultural Research Council.*)

I. Experiments in 1936.

Preliminary investigations on the problem of affording protection to the potato plant growing in soil infested with cysts of *Heterodera schachtii*, were carried out by Hurst & Triffitt (1935, i, ii, 1937). In these experiments promising results were obtained by the application of calcium cyanamide or of ferric oxide to the soil, and trials with these chemicals were designed on a larger scale. A small field experiment (Hurst & Franklin, 1937) was carried out in Lincolnshire during 1936, using dressings of 30 cwt. per acre of calcium cyanamide and 12 cwt. per acre of ferric oxide. On the cyanamide plots an increased yield of potatoes was accompanied by a relatively small increase in the cyst population of the soil. With ferric oxide the results were not significantly different from those on the untreated control plots.

Infected land in Bedfordshire was rented by the Institute for experimental work in May, 1936. The cropping for the previous four years had been: early potatoes 1932, beet 1933, peas 1934 and early potatoes 1935. "Potato sickness" was much in evidence during 1935. The soil was a light loam, with a low pH of approximately 5·0 and a correspondingly high lime requirement of several tons per acre. The organic nitrogen and potash contents were rather low and the phosphate content normal.

Two 6 × 6 Latin squares were marked out, the area of each plot being approximately 1/40th acre. Various metallic oxides were applied on the

first Latin square as follows: control, B.D.H. precipitated ferric oxide (10 cwt. per acre), commercial "levigated" ferric oxide (10 c.p.a.), crude commercial ferric oxide (50 c.p.a.), iron powder (6 c.p.a.), and a high quality of commercial zinc oxide (6 c.p.a.). The chemicals were applied to the sides and bottoms of the furrows after the "seed" potatoes had been placed in position. The crude ferric oxide was divided into two portions. The larger of these, corresponding to 40 c.p.a., was incorporated with the soil by means of a rototiller. The remaining 10 c.p.a. was dusted along the rows at the time of planting. The iron powder, because of its small volume, was concentrated in the region of each potato.

On the second Latin square dressings of calcium cyanamide equivalent to 0, 10, 20, 25, 30 and 40 c.p.a. were applied. The plots were rototilled to a depth of 6 to 9 ins., and were then left for a period of three weeks to allow the chemical to decompose beyond the stages at which it might be toxic to the potato plant. The soil-moisture content at the time the chemical was applied was 13·4%, and three weeks later 18·9%.

King Edward seed potatoes were planted in June, the distance between the setts being 1 ft. 6 ins., and between the rows 2 ft. 5 ins. The artificial was "Humber Fish Manure," applied at the rate of 10 c.p.a. a few days before planting. The months of June and July were abnormally wet and, except on the plots heavily dressed with cyanamide, difficulty was experienced in keeping down the growth of weeds. It could be seen, however, that the plants on the control plots of the first Latin square were at least as large and healthy as those on the ferric oxide plots, whilst those on the zinc oxide plots were the poorest. On the plots which received 40 c.p.a. of cyanamide there was early retardation in growth, but later the plants on these and most of the other plots treated with cyanamide were much superior in appearance to those on the controls. Individual plants were dug from the 40 c.p.a. plots during July, and examination of the roots usually revealed the presence of a very few cysts, whereas plants from the control plots were relatively heavily infested.

During August the plants were subjected to a very severe attack of blight and died down towards the end of the month. The growing period was thereby restricted to less than three months, and in consequence the yields were small and of interest only for purposes of comparison.

Metallic Oxides

(Latin Square No. 1).

Yields.—The yields from the plots treated with iron and zinc oxides are shown in Table I. The yield following treatment with levigated ferric oxide was not significantly different from that of the control plots, but with each of the other treatments it was considerably less. It appears probable that the acidity of this soil (*p*H 5·0) rendered the metallic oxides sufficiently soluble to be absorbed to a small degree by the growing plant. This might account for the injurious effect of zinc oxide. There is, however, no obvious reason why precipitated ferric oxide should have given a lower yield than the levigated product.

Table I

Yields (lbs. per plot)

Control	Levigated Fe_2O_3	Ppted Fe_2O_3	Crude Fe_2O_3	Iron Powder	Zinc Oxide	Standard Error
57·3	52·7	41·5	27·7	34·3	23·2	3·05

Cyst counts.—Soil samples to a depth of 9 ins. were taken with a cylindrical soil-sampler, before and after the crop. From each plot, eight individual samples were taken to make a bulk sample. Cyst counts were carried out on 50-gram portions of air-dried soil, the procedure being that described by Hurst & Franklin (1937). Five counts were made on each sample, and the figures given in Table II for each plot are the averages of the five counts. The averages per treatment of six plots quoted in the Table are therefore averages of 30 counts. The largest increases in numbers of cysts were found on the levigated and precipitated ferric oxide plots, in spite of the smaller yield and presumably smaller root development of the plants as compared with the controls. There appeared to be some evidence that these two chemicals possessed the property of attracting hatched larvae, since many tubers were observed on which numerous cysts had developed, whereas no such tubers were found on the control plots. It is possible that, if this property of attraction existed, it was due to the concentration of potato root excretion adsorbed by the powder.

TABLE II.

Treatment	Plot No.	Yield (lbs.)	Cysts per 50 gm. soil		Increase in cyst count
			Before crop	After crop	
Control	5	60	73	81	+ 8
	9	46	54	46	− 8
	18	64	64	73	+ 9
	19	78	74	77	+ 3
	26	56	56	48	− 8
	34	40	60	99	+39
	Average ...	57·3	63·5	70·7	+ 7·2
"Levigated" Fe_2O_3	1	47	62	69	+ 7
	11	58	77	77	0
	16	41	66	82	+16
	24	59	75	88	+13
	27	56	49	52	+ 3
	32	55	85	116	+31
	Average ...	52·7	69·0	80·7	+11·7
Precipitated Fe_2O_3	2	41	56	66	+10
	12	42	68	74	+ 6
	13	49	80	74	− 6
	22	50	37	46	+ 9
	29	36	46	57	+11
	33	31	63	96	+33
	Average ...	41·5	58·3	68·8	+10·5
Crude Fe_2O_3	6	45	62	53	− 9
	8	20	61	64	+ 3
	17	17	62	73	+11
	21	21	57	49	− 8
	28	27	42	62	+20
	31	36	85	108	+23
	Average ...	27·7	61·5	68·2	+6·7
Iron Powder	4	24	70	53	−17
	7	42	67	46	−21
	15	28	74	71	− 3
	20	33	59	61	+ 2
	30	43	83	93	+10
	35	36	45	67	+22
	Average ...	34·3	66·3	65·2	−1·1
Zinc Oxide	3	19	72	59	−13
	10	19	79	78	− 1
	14	25	73	36	−37
	23	23	60	66	+ 6
	25	28	78	95	+17
	36	25	41	54	+13
	Average ...	23·2	67·2	64·7	− 2·5

Cyst dissections.—The cysts separated from a soil sample of each plot were dissected under the binocular microscope and their contents of apparently viable eggs estimated in the way described by Triffitt (1934). The percentage "fulness" of the cysts was calculated and an average for the six plots of the same treatment obtained. This, multiplied by the average number of cysts per plot, gave the equivalent number of full cysts contained in 50 grams of soil. These figures are shown in Table III, and in spite of the large experimental error in the method used, the increases tabulated are probably more significant than those of Table II.

In view of the failure of ferric oxide, on this type of soil at least, experiments with it have been discontinued.

TABLE III.

Cyst dissections.

Treatment	Equivalent number of full cysts per 50 gm. soil.		
	Before crop	After crop	Increase
Control	34·7	40·2	+ 5·5
"Levigated" Fe_2O_3	37·7	49·2	+11·5
Precipitated Fe_2O_3	33·8	43·0	+ 9·2
Crude Fe_2O_3	35·1	37·9	+ 2·8
Iron powder	38·8	39·4	+ 0·6
Zinc Oxide	37·1	35·1	− 2·0

CALCIUM CYANAMIDE

(Latin Square No. 2).

Yields.—The yields obtained from the plots treated with calcium cyanamide are shown in Table IV. It will be seen that the plants responded to each of the treatments and that the highest yields were obtained following dressings of 20 and 25 c.p.a. It is possible that the margin between these and the yields from 30 and 40 c.p.a. would have been smaller if the plants had continued their growth for a normal length of time. Moreover, during the early period after planting, growth was much retarded on the heavily treated plots, and it appears very probable that higher yields would have been obtained if the chemical had been

applied earlier than three weeks before planting. The optimum time to allow between applying the chemical and planting potatoes would, for maximum yield, depend upon several factors, chief of which would normally be the amount of the dressing and the degree of soil moisture. These arguments are concerned almost entirely with the properties of calcium cyanamide as an artificial fertiliser.

TABLE IV.

Yields (lbs. per plot).

Control	Calcium cyanamide					Standard Error
	10 c.p.a.	20 c.p.a.	25 c.p.a.	30 c.p.a.	40 c.p.a.	
47·7	79·5	123·0	122·8	103·0	96·0	8·3

Cyst counts.—The cyst counts on samples from each plot are shown in Table V. It will be noticed that the cysts were distributed very unevenly on this land. Thus, for example, the cyst counts before the crop on the adjacent plots numbers 61 and 62 were, respectively, 105 and 16. There was, however, no correlation on plots which received the same treatment, (a) between yield and cyst count before the crop, or (b) between yield and increase in the cyst count as a result of the crop.

The results indicate that only on the plots which received the heavy dressing of 40 c.p.a. was new cyst formation restricted to a very small number, and it is clear that thorough mixing of the chemical with the soil had not been obtained.

Cyst dissections.—The results of cyst dissections are given in Table VI, and these show that the increases in the equivalent numbers of full cysts are inversely proportional to the amount of the cyanamide dressing. There is, of course, no indication as to how many of the apparently viable eggs have been killed by the lethal action of cyanamide when in sufficient concentration. It is clear, however, that the amount of new cyst formation on the plots which received 40 c.p.a. was extremely small.

TABLE V.

Treatment	Plot No.	Yield (lbs.)	Cysts per 50 gm. soil		Increase in cyst count
			Before crop	After crop	
Control	39	66	88	93	+ 5
	46	49	45	51	+ 6
	50	44	13	61	+48
	59	62	74	101	+27
	66	38	125	155	+30
	67	27	98	120	+22
	Average ...	47·7	73·8	96·8	+23·0
$CaCN_2$ 10 c.p.a.	40	69	47	94	+47
	45	62	25	46	+21
	53	96	36	75	+39
	56	90	12	38	+26
	61	72	105	106	+ 1
	72	88	99	101	+ 2
	Average ...	79·5	54·0	76·7	+22·7
$CaCN_2$ 20 c.p.a.	42	127	105	146	+41
	43	143	28	41	+13
	52	111	30	56	+26
	57	98	18	53	+35
	65	122	34	63	+29
	68	137	55	59	+ 4
	Average ...	123·0	45·0	69·7	+24·7
$CaCN_2$ 25 c.p.a.	41	106	102	125	+23
	48	87	59	96	+37
	49	167	19	39	+20
	58	129	22	56	+34
	62	100	16	30	+14
	69	148	19	32	+13
	Average ...	122·8	39·5	63·0	+23·5
$CaCN_2$ 30 c.p.a.	37	113	53	61	+ 8
	44	109	23	34	+11
	51	80	29	59	+30
	60	72	142	137	− 5
	64	122	32	50	+18
	71	122	41	45	+ 4
	Average ...	103·0	53·3	64·3	+11·0
$CaCN_2$ 40 c.p.a.	38	104	56	51	− 5
	47	100	55	71	+16
	54	76	89	87	− 2
	55	107	19	21	+ 2
	63	77	30	30	0
	70	112	34	48	+14
	Average ...	96·0	47·2	51·3	+4·1

TABLE VI.
Cyst dissections.

Treatment	Equivalent number of full cysts per 50 gm. soil.		
	Before crop	After crop	Increase
Control	22·8	35·0	+12·2
$CaCN_2$ 10 c.p.a.	19·3	40·8	+21·5
,, 20 ,, ...	15·5	34·2	+18·7
,, 25 ,, ...	13·8	27·8	+14·0
,, 30 ,, ...	17·5	26·4	+ 8·9
,, 40 ,, ...	22·4	21·6	— 0·8

Hatching from Cysts.—For the comparison of rates of hatching, soil samples were kept until the spring of 1937. The cyst contents of five samples from each treatment were placed separately in Petri dishes and subjected to the stimulus of potato root excretion. The average numbers of larvae which hatched during four weeks are given in Table VII.

TABLE VII.
Rates of hatching.

Treatment	Number of hatched larvae (Average of five samples).				
	1st week	2nd week	3rd week	4th week	Total
Control	442	373	413	20	1,248
$CaCN_2$ 10 c.p.a. ...	822	402	304	52	1,580
,, 20 ,, ...	713	175	248	31	1,167
,, 25 ,, ...	522	179	357	39	1,097
,, 30 ,, ...	534	185	464	32	1,215
,, 40 ,, ...	488	113	287	24	912

The results show that even the dressing of 40 c.p.a. was not lethal to many of the cysts. In view of the very small increase in numbers of cysts on these plots, the action of the cyanamide must in part be interpreted as a very strong retardation of hatching of larvae during plant

growth. This incomplete action must be attributed to uneven distribution of the chemical with the soil, since we have demonstrated in pot experiments with this soil that when a dressing of calcium cyanamide equivalent to 40 c.p.a. was intimately incorporated, it was lethal to all the cysts.

Potatoes were grown again on these plots during 1937.

II. Experiments in 1937.

The results with metallic oxides on this type of soil were discouraging, and these experiments were not continued. The results with calcium cyanamide were promising and potatoes were grown for a second year, the plots receiving no further treatment other than artificials.

In addition an experiment was arranged in which varying amounts of cyanamide were applied to the soil in the autumn preceding the planting of potatoes. In this way, several months would elapse, during which all or most of the cyanamide would be converted to nitrates, and partially leached from the soil. It was not anticipated that in these circumstances there would be any retardation of plant growth, even following the heavy dressing of 40 cwt. per acre. Differences in yield would presumably be more attributable to the lethal action of cyanamide on *H. schachtii* larvae, than to its property as a fertiliser. For comparison as an artificial fertiliser, a treatment with ammonium sulphate plus lime was included. In view of the very acid nature of this soil, lime was also applied to the control plots. New land was not available and these experiments were therefore carried out on the plots previously treated with metallic oxides. It was thought that the possibility of any residual effect of ferric oxide, particularly after rototilling the land, was remote. In the case of zinc oxide, the chance was taken that residual effect would be inappreciable. The experiment was therefore designed for a 6×6 Latin square with the following treatments: (A) control + an amount of hydrated lime with Ca content equivalent to that in calcium cyanamide, 20 c.p.a., (B) ammonium sulphate + hydrated lime, equivalent in N_2 and Ca content to calcium cyanamide, 30 c.p.a., (C) $CaCN_2$, 20 c.p.a., (D) $CaCN_2$, 25 c.p.a., (E) $CaCN_2$, 30 c.p.a., and (F) $CaCN_2$, 40 c.p.a.

The land was in a good state of cultivation when the above chemicals were applied during the first week in December, 1936. The soil-moisture was 17·8% and no rain fell during this week ; conditions favourable to the lethal action of cyanamide. The chemicals were spread evenly on the surface and the plots rototilled twice to a depth of fully 9 ins. The mixing was clearly more complete than that on the other Latin square in the spring of 1936. In the case of the ammonium sulphate plus lime plots, the lime was first mixed with the soil by rototilling, followed by ammonium sulphate and a second rototilling.

Both Latin squares were prepared for the planting of Majestic seed potatoes towards the end of April, 1937. An artificial fertiliser consisting of ammonium sulphate, superphosphate and potash in the ratio 2 : 3 : 3, was applied at the rate of 12 c.p.a. the day before planting. Seed was planted on April 28th, the distance between the setts being 1 ft. 6 ins., and between the rows 2 ft. 4 ins.

As anticipated, there was no retardation in early growth on any of the cyanamide plots. On the first Latin square the plants on the control plots showed very marked symptoms of " potato sickness ", and the difference in appearance between these and plants on the treated plots was very striking (see Plate). It could be seen also that plants on the 40 c.p.a. cyanamide plots were the largest and healthiest, and that those on the ammonium sulphate plots were inferior to those on any of the cyanamide plots.

On the second Latin square the difference was much less noticeable and the plants on some of the control plots did not show marked symptoms of " potato-sickness." In general the plants on the treated plots which had received 20, 25, 30 and 40 c.p.a. of cyanamide appeared better than those on the control or 10 c.p.a. plots, but individual plots could be seen where this was not the case. The 40 c.p.a. plots were not conspicuous, as was the case during 1936.

The plants died down towards the end of September, and the crop was then " lifted."

TABLE VIII.

First Latin Square.

B 125 (98)	E 249 (90)	D 246 (88)	C 274 (96)	A 31 (73)	F 308 (95)
C 194 (98)	F 420 (92)	A 69 (86)	B 142 (103)	E 260 (114)	D 249 (103)
F 311 (98)	D 308 (92)	B 224 (70)	E 284 (159)	C 192 (95)	A 60 (88)
E 207 (116)	A 52 (86)	F 387 (116)	D 277 (148)	B 103 (56)	C 188 (100)
A 70 (78)	C 170 (106)	E 319 (91)	F 467 (125)	D 287 (95)	B 215 (80)
D 250 (124)	B 146 (78)	C 280 (110)	A 88 (86)	F 389 (86)	E 359 (31)

Yield in lbs. of " ware " and (in parentheses) " seed + chats."

A = Control + Lime.
B = Ammonium sulphate + Lime.
C = Calcium cyanamide 20 c.p.a.
D = " " 25 "
E = " " 30 "
F = " " 40 "

TABLE IX.

		Control + Lime	Ammonium sulphate + Lime	Calcium cyanamide				Standard Error
				20 c.p.a.	25 c.p.a.	30 c.p.a.	40 c.p.a.	
Total Yield	Lbs. per plot	144·5	240·0	317·2	377·8	379·8	482·3	14·8
	Tons per acre	2·7	4·5	6·0	7·1	7·1	9·1	0·28
" Ware " only	Lbs. per plot	61·7	159·2	216·3	269·5	279·7	380·3	15·6
	Tons per acre	1·2	3·0	4·1	5·1	5·3	7·1	0·29

TABLE X.

Second Latin Square.

E 142 (38)	F 182 (60)	A 185 (66)	B 196 (129)	D 259 (86)	C 188 (207)
C 166 (65)	E 135 (72)	B 177 (100)	A 308 (100)	F 213 (101)	D 233 (101)
D 219 (60)	A 200 (70)	E 358 (82)	C 295 (112)	B 151 (93)	F 233 (114)
F 392 (82)	B 184 (100)	C 450 (86)	D 238 (95)	A 192 (147)	E 329 (120)
B 201 (76)	D 244 (103)	F 354 (92)	E 220 (95)	C 175 (127)	A 193 (101)
A 88 (90)	C 164 (72)	D 368 (105)	F 206 (85)	E 270 (100)	B 166 (100)

Yield in lbs. of "ware" and (in parentheses) "seed + chats."

A = Control.
B = Calcium cyanamide 10 c.p.a.
C = ,, ,, 20 ,,
D = ,, ,, 25 ,,
E = ,, ,, 30 ,,
F = ,, ,, 40 ,,

TABLE XI.

		Control	Calcium cyanamide					Standard Error
			10 c.p.a.	20 c.p.a.	25 c.p.a.	30 c.p.a.	40 c.p.a.	
Total Yield	Lbs. per plot	290·0	278·8	351·2	351·8	326·8	352·3	11·2
	Tons per acre	5·2	5·0	6·3	6·3	5·8	6·3	0·20
"Ware" only	Lbs. per plot	194·3	179·2	239·7	260·2	242·3	263·3	25·9
	Tons per acre	3·5	3·2	4·3	4·6	4·3	4·7	0·46

The yields from the first Latin square are shown in Tables VIII and IX. The total yield of 9·1 tons per acre following treatment with 40 c.p.a. of cyanamide, compared with the yield of 2·7 tons per acre from the control plots, is remarkable and, in view of the fact that five months elapsed between applying the chemical and planting potatoes, it is unlikely that the difference can be explained by the fertiliser properties of cyanamide. This appears to be confirmed by the yield from the ammonium sulphate plots, which received an amount of nitrogen equal to that contained in the 30 c.p.a. dressing of cyanamide, since this yield is significantly less than that obtained from even the 20 c.p.a. cyanamide plots. Since, however, with these very large dressings it might conceivably be argued that calcium cyanamide retains its properties as a fertiliser for a longer period than ammonium sulphate, it is proposed to carry out an experiment on land free from *H. schachtii* infection for a comparison of these two substances, applied in the autumn at the rate of 40 c.p.a. The comparison will also be made on nearby infected land with soil of the same type.

The yields from the second Latin square are shown in Tables X. and XI. The amounts and similarity of the yield from plots which in the previous year had been treated with 20, 25, 30 and 40 c.p.a. of cyanamide confirm the results of the hatching experiment, namely, that the chemical had exerted a temporary retarding effect on the hatching of larvae from many of the cysts. The yields per plot following these four treatments, considered as a whole, are significantly larger than those from control plots or cyanamide plots which received 10 c.p.a., and this will be considered when soil samples have been examined.

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Control plot.

Adjacent 40 c.p.a. Cyanamide plot.

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New Records of Helminths in British Birds.

By PHYLLIS A. CLAPHAM, Ph.D.
(*Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

THE object of this short paper is to record a number of new host species for certain common helminths of birds. These new records show that the host range of some helminths is not so circumscribed as had been previously believed. Most of these parasites have been obtained at routine post-mortem examinations and hence were the result of natural infections in the field. A few however were the result of experimental feedings at this Institute.

Davainea proglottina, a parasite of the domestic fowl and lately shown to be a parasite of certain other gallinaceous birds has been found in the pigeon, *Columba livia*, three parasitized specimens being shot in Hertfordshire. In one case the infestation was heavy and was associated with a considerable degree of enteritis and some small haemorrhages. The other 2 pigeons had only light infections. The first bird was also infested with *Raillietina tetragona*, 2 fully mature specimens being obtained from the small intestine. The intermediate hosts—snails and slugs—have not yet been seriously considered as food of the pigeon.

Anomotaenia microrhyncha, a dilepid cestode usually associated with plovers has been recovered from a red legged partridge, *Alectoris rufa* from Wantage in Berkshire. There were present a very large number of specimens, all showing genitalia but none of which were quite gravid. The genera *Arion* and *Limax* have been implicated as vectors of one species of this genus.

Among Trematodes there are only 2 new records to note. The first is that of *Echinostoma revolutum*, 5 mature specimens of which were found in a partridge, *Perdix perdix* from the Norfolk district of England. A small haemorrhagic area was noted at the points of attachment. A pheasant, *Phasianus colchicus* from Warwickshire was found to be parasitized with 2 *Harmostomum commutatum*, a parasite of the hen and pigeon. Land molluscs here too would seem to be vectors of this genus.

Among Nematodes *Trichostrongylus tenuis* has been found as a natural parasite of the chukor, *Alectoris chukor*, turkey, *Meleagris gallopavo* and the guinea fowl, *Numida meleagris*. The turkey was one of a flock of

7 birds living at this Institute which at death was found to contain, among other helminths, nearly 2,000 *T. tenuis* in its caeca. Though this was an appreciable infection, there were no lesions to be described beyond some slight venous congestion. The cause of death was a massive infection with *Ascaridia lineata*, the larvae of which had done considerable damage to the mucosa of the small intestine. The guinea fowl had only a light infection and was showing no ill effects. Both these birds were confined to a small area, to which the infection must have been brought accidentally from the outside, as no part of the Institute was then seeded down with *T. tenuis* eggs or larvae experimentally. The infection was probably introduced by wild birds. There are a very few partridges in the neighbourhood, but large numbers of rooks which in their daily migrations, doubtless settle in partridge country. The infection of *A. chukor* is not surprising as it has recently been shown from this laboratory that it is a frequent parasite of *A. rufa*, the common red legged partridge and a near relative. It was a light infection—less than 100 worms—and there were none of the symptoms usually associated with partridge disease.

Capillaria longicollis, a parasite very widespread geographically in a great variety of hosts, has been recovered from the small intestine of the domestic pigeon and from *A. chukor*. That these were not accidental infections is shown by the fact that it has occurred in 7 pigeons and in the only 2 chukors examined.

Ascaridia compar, recently recorded as a parasite of the red-legged partridge is now shown to be a parasite of *A. chukor* also. One bird contained 47 and the other 18 specimens. These two birds also harboured *Heterakis gallinae*, as did 3 Brush Turkeys, *Alectura lathami*, from Whipsnade Park.

Acanthocephala have not previously been much recorded from game birds but recently natural infections of partridge, *Perdix perdix* in Norfolk and pheasant, *Phasianus colchicus* in Hertfordshire with *Prosthorhynchus transversus* have been observed at post-mortem examinations. It has furthermore been possible to transmit this parasite to chickens experimentally by seeding down a small plot of land with eggs taken from these worms. The parasite occurs naturally in the small intestine of various wild birds where it can cause symptoms of inflammation and enteritis. The vector is not known.

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Are there Host Strains within the Species of *Syngamus trachea* ?

By Phyllis A. Clapham, Ph.D.

(*Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

In 1928 Taylor put forward the theory that " . . . *Syngamus trachea* is a species within which there may be found a number of strains showing a peculiar physiological adaptation to the host in which they occur." He had not had much success in a number of experiments in which he had tried to infect chickens with *S. trachea* from starlings. The first experimental feedings resulted in only light infections though control experiments with the chicken " strain " resulted in much heavier ones. After the first passage through the chicken however, the gapeworms had become more adapted to the new host, and could produce much more serious infections in the next generation. There were other differences to be observed also—the rate of growth was slower, the proportion of larvae which developed was smaller in the starling " strain " and the final position in the definitive host was different : the Syngamus from the starlings attached itself to the upper part of the trachea while the chicken worms were found in the lower half. He suggested therefore that it is highly improbable that this " strain " is likely to be harmful to chickens and this assumption that the starling population of a farm can be ignored as far as " gapes " is concerned has been adopted in the pamphlets issued by the Ministry of Agriculture.

In 1934 it was shown from this laboratory that *Syngamus trachea* occupies a unique position among helminths in that it may complete its life-history either directly or indirectly by means of an intermediate host. Using an intermediate host, in this case earthworms, much heavier infections could be obtained in the definitive host. Taylor (1935) showed that various land molluscs may also act as vectors but he does not seem to have compared numerically the infections resulting from the direct and the indirect methods.

By means of the earthworm *Helodrilus* (syn. *Eisenia*) *foetida*, Syngamus was transmitted to chickens from pheasants, partridges and rooks (1934) and the following year the starling " strain " was successfully used in

producing gapes in chickens in their first passage. *S. merulae* from the blackbird was also transmitted by this method.

During the past four years, a large variety of birds have been examined for helminths and gapeworms have been recovered from a number of species from which observations have been made. Recently four species of wild birds have yielded up a small number of worms which have been used for experiments.

Technique.

The method of culturing has been the same throughout. The worms were removed from the trachea, teased up in tap water and allowed to develop in Petri dishes at laboratory temperatures. Meanwhile earthworms were obtained from a greenhouse from which birds were excluded and were kept in soil sterilized by heating to 60°C. This temperature effectively kills all helminth eggs. The soil was changed twice at intervals of two days after which the infective eggs of *Syngamus trachea* were added and the earthworms were allowed to become infected under natural conditions. Control earthworms lived in sterile soil. The chickens were infected by feeding these *Helodrilus foetida* not earlier than 14 days after they (the earthworms) had been exposed to infection, and they were examined 14 days afterwards unless they died before.

The chickens were all incubator hatched R.I.R. × L.S. cockerels. The young pheasants and partridges were hatched at this Institute in an incubator and kept on a lawn from which domestic birds were excluded. Though there were a number of wild birds about, none of these experimental birds ever became infected naturally. The wild birds did not congregate here owing to an abundance of food elsewhere.

The gapeworms were obtained from the pheasant, partridge, jackdaw, crow, jay and magpie. In the case of the last four species, as only a limited quantity of material was available, the number of experimental animals was small.

Experiments.

Plenty of material was obtained from pheasants and partridges and this was found to be mutually infective—2 partridges and 7 pheasants were infected, all of which developed clinical symptoms. One pheasant died of asphyxiation 12 days after infection.

In the early summer of this year, 4 young crows, *Corvus corone*, were shot and they were found to contain between them 21 gapeworms. From this stock 2 pheasants were infected with 4 and 7 worms and a partridge with 6 worms respectively. Three chickens took the infection also, 2 birds having 3 and the other 14 worms in the trachea. Two jays *Garrulus glandarius*, which were slaughtered locally contained 3 pairs of gapes from which experimental infections of 2 pheasants and 1 partridge were effected: each bird developing 2 pairs of worms. A jackdaw, *Coloeus monedula*, was also found to be infected with gapeworms, there being 3 specimens by means of which 2 chickens and 2 pheasants were infected, there being a total of 7 worms resulting from this experiment. Finally a magpie, *Pica rustica*, which contained 2 pairs of gapeworms was the source of experimental infections in 2 chickens and 1 pheasant. In this case the chickens developed 3 pairs of worms between them and the pheasant had 2 pairs.

It is admitted that the number of experimental animals is small, owing to the paucity of parasitic material but the fact that 100% infections resulted from the feedings seems to show that there is no difficulty about causing an infection in economically important birds. If a physiological difference exists, passage through the earthworm would seem to remove it.

There is now experimental evidence that the rook and crow, jackdaw, magpie, jay, starling, turkey, pheasant and partridge "strains" are transmissible to other birds and are liable to set up disease there when the indirect life-cycle, using an intermediate host, is adopted. This is probably the normal method of transmission under natural conditions, as Taylor has shown the larvae can remain infective in the earthworm up to a period of three-and-a-half years and that the earthworms are important storage agents of the larvae. Infective eggs and free larvae in the soil have only a limited length of life. As certain of these birds, notably the rook, crow and starling are extraordinarily abundant locally and often frequent feeding grounds in vast numbers, it may well be that they are the causes of some of the unexplained outbreaks among chicks of domestic and game birds which are reported from time to time, as Lewis has already suggested.

Furthermore *Syngamus trachea* has also been recorded from many other wild birds, the transmissibility of which there has been no opportunity to investigate up-to-date, but it seems not unreasonable to suppose

that these also may develop in other birds without much difficulty. Hence again wild birds may be of major importance in the spread of the disease.

Though *S. merulae* was shown earlier (1934) to be infective to chickens under experimental conditions, this species does not seem to be so abundant that it need be considered as of great potential importance.

As to the position that these helminths have taken up in the trachea, not much is yet known. Starling gapes have a tendency to attach themselves at the buccal end of the trachea. In the rooks the worms were scattered all over the whole length; indeed they are often so numerous that it would be impossible for them to be localized in any one small area. The worms obtained from the crows used the whole length also—a total of 7 was obtained from the upper third, 5 from the middle and the rest of them had attached themselves to the pulmonary ends of the trachea. In the resulting experimental infections, the worms showed no predilection for any one region. In the case of the other birds, there was not sufficient material to make any judgment.

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The Relation of Helminthiasis to Leukaemia in Domestic Fowls.

By Phyllis A. Clapham, Ph.D.

(*Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

During the past year about 50 domestic hens from a farm at Wheathampstead, Herts, have been sent to this laboratory for post-mortem examination. The birds came in singly at first. Later as the helminthic fauna seemed likely to prove interesting, they were kindly sent in groups of three. When a diseased bird died, a thin one and an apparently healthy one were slaughtered so as to effect a control. The hens had been bought as adults from a number of local farmers and were kept outside on free range. They belonged to the breeds Ancona, Light Sussex, Rhode Island Red or were a cross between R.I.R. and Light Sussex. All the birds seemed to be equally affected.

The birds were reported as having been picked up dead, no particular symptoms having been noticed. When this had occurred a few times, the rest of the flock was examined periodically and occasionally thin ones were noticed. These invariably died within a short time if they were not previously slaughtered.

Post-mortem examination of the diseased birds always showed an advanced stage of leukaemia. The lesions were practically identical in each case, varying only in degree. The liver and spleen were hypertrophied and were riddled with the white spots characteristic of this disease. The ovary and heart were frequently affected and in one very severe case the kidneys, and pancreas, together with the whole of the gut and mesenteries also. A few cases showed a greyish discoloration of the iris and some, but not all, showed hypertrophy of the sciatic and/or brachial nerves. There had been a report of paralysis or lameness in a few living birds. Intense emaciation was a constant feature in all. In the thin birds which were slaughtered the same lesions occurred but were less pronounced. The healthy controls showed no pathological changes.

A certain number of the hens had died from causes other than leukaemia. Most of them were egg bound, a few had had an accident and some had pneumonia. One had a duodenal ulcer which will be referred to later.

The helminth fauna of all these birds was examined and accurately counted. The healthy birds which had been killed for food acted as controls and their worm burden considered as " normal " for this farm. These will be considered first.

Heterakis gallinae was the most frequent and most abundant parasite encountered. Of the normal birds two were without any, three contained 67, 127 and 188 respectively ; the rest contained less than 20. *Capillaria longicollis* occurred 3 times, there being 4, 8 and 23 specimens, while in 4 birds there were 2, 4, 4 and 3 *Ascaridia lineata*. One bird had a few isolated segments of a species of *Raillietina*. All these helminths were mature.

Turning next to the hens which had died from a cause other than leukaemia, we find slightly heavier infestations with helminths. The numbers of *H. gallinae* varied from 51–200, *A. lineata* from 2–37 and *Capillaria* from 4–73, except in one case where there were nearly 200 associated with an ulcer in the duodenum. All these worms also had reached maturity.

In sharp contrast to these two sets of results were the leukaemia birds. In those which had been found dead, there were never less than 1,572 *H. gallinae* and the number rose to over 4,000 countable forms. Actually there were more, for there were a large number of very small free larvae in close contact with the mucosa. Some of these and of those free in the lumen of the gut may easily have been so small as to have been lost in the sieving and cleaning of the débris. *Ascaridia* numbers varied from 198–247 and *Capillaria* from 27–nearly 500. All the birds invariably harboured all three species. *Trichostrongylus tenuis* was also found.

These high infestations associated with leukaemia cannot be ignored and indeed they have been frequently remarked upon in the literature. One very striking fact which has not yet been commented upon has, however, been noticed in these post-mortems. Most of the worms removed from diseased birds were very young adult or immature forms. To quote a few specific examples : there were associated 6 adult and over 2,000 young *H. gallinae* in one bird ; in another, 13 adults and 2,141 young ones. Similar observations were made with the other two common species. With *Ascaridia* 3 adults and 60 young ones were found together or 4 adults with 94 young. The mucosa of the small intestine usually showed evidence of heavy larval infestation.

In the past various workers have put forward the theory that leukaemia and perhaps paralysis are caused by helminths, but this theory is not now generally accepted. The present writer would like to suggest however that there is considerable circumstantial evidence that the presence of the leukaemia virus may pre-dispose the host to helminthic infestation. Leukaemia takes from 6–8 weeks to run its course and during the latter part of the time the animal would presumably be feeling the full bad effects.

The young forms of *Heterakis* and *Ascaridia* have been examined carefully and in the light of the results of previous workers (Clapham, 1933 ; Ackert, 1931 and Roberts, 1936 & 1937) their age can be established with a fair degree of accuracy. It has been estimated in this way that the larvae and young forms found in such vast numbers in the gut are not more than 3 weeks old and many are younger. Information on *Capillaria* is not forthcoming, but there is no reason to suppose that a different state of affairs exists there. We cannot believe that for the three weeks immediately preceding death, the chicken suddenly picks up an increasingly large number of infective stages and larvae. Indeed, the lack of such forms in the controls contradicts this possibility, therefore we must assume that a larger number of larvae than normal are able to develop within the body of the host. Heavy infections are not found in those birds which had died of being egg-bound, of pneumonia or of other causes. It is not unreasonable to assume therefore that the presence of the leukaemia virus has resulted in a lowering of the helminthic resistance. The mechanism of this is not obvious at the present moment. It is possible that any other chronic disease, such as tuberculosis, might have the same effect, the long course of the disease giving time for the break-down of resistance. There is another possible, though improbable, explanation that the development of the helminths is in some way inhibited by the leukaemia virus. Against this, however, is again the very large number found present in the gut.

Another piece of evidence that supports this theory of lowered helminth resistance is the fact that *Trichostrongylus tenuis* has been recovered from the leukaemia hens and not from the others. This nematode is normally a parasite of grouse and partridges and though it has been transmitted experimentally to a number of other hosts, it is not considered of any economic importance except to game. It has been transmitted in this laboratory to the domestic fowl, but this would seem

to be the first record of its natural occurrence in *Gallus gallus*. The possibility that this parasite may at some future time become of economic importance in this host must not be overlooked as pathological lesions regularly occur in its normal habitat and have been induced in the experimentally infected birds. The specimens of *T. tenuis* found in this series of examinations were always mature, but this fact in no way conflicts with the observations cited above as it has been shown by experiments that they reach the gravid stage as early as 7 days after entry into the body. The farm from which these birds came does not lie in the midst of good shooting country. There are a number of coveys about and they presumably have carried the infection as they have flown over or they may have come down for feeding with the hens, though this has never been noticed by the farmer. The farm cannot be heavily infected as no other birds carried the parasite, though they are potential hosts. This is further evidence that the resistance of the leukaemia birds to helminthic infestation has been extensively reduced.

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On the Relative Distribution of Cysts of *Heterodera schachtii* and a Chemical Dressing incorporated with Infected Land by means of a Rototiller.

By Richard H. Hurst, B.Sc., Ph.D., F.I.C.

(*Senior Research Assistant for Biochemistry, Institute of Agricultural Parasitology, St. Albans.*)

A FIELD experiment was commenced in Bedfordshire during 1937, when soil infested with cysts of *Heterodera schachtii* was treated with very heavy dressings of calcium cyanamide. For comparison, a treatment with ammonium sulphate and lime was included. The lay-out was in the form of four randomised blocks of five treatments: control, calcium cyanamide 40, 60 and 80 c.p.a., and ammonium sulphate 80 c.p.a. plus hydrated lime 63 c.p.a. The last dressing contained the same amounts of nitrogen and lime as 80 c.p.a. of calcium cyanamide.

The chemicals were spread on the surface of the soil and mixed with it by means of a No. 30 Rototiller. This was used twice on each plot, the first time to about half the depth it was capable of reaching, *i.e.*, 4 to 5 ins., and the second time to the full depth of 8 to 9 inches. The experiment was carried out in a corner of the field and although the surface soil was of a light sandy nature, the subsoil in parts at a depth of about 6 ins. was of a hard clayey nature. For this reason the mixing by rototilling may not have been carried out as efficiently as in some previous experiments. In the case of the treatment with ammonium sulphate and lime these chemicals would normally, to prevent escape of nitrogen, be mixed separately with the soil. It has been found in laboratory experiments, however, that ammonia in sufficient quantity is lethal to *H. schachtii* larvae. It was decided, therefore, to spread these chemicals successively on the surface of the soil, and to rototill immediately afterwards. It was thought that the more intimate mixing of the two chemicals obtained by this method would, for the purpose of exerting any lethal action on the eggs contained in the cysts, more than compensate for the escape into the atmosphere of some of the nitrogen as ammonia vapour.

The plots were left for nine days, during which time no measurable rain fell. Soil samples were then taken by means of a 9 in. cylindrical soil-sampler fitted with a hinge to allow it to be opened out after it had been filled with soil. The soil was removed from the sampler in successive portions of 1½ins., so that six samples were thus obtained corresponding with varying depths beneath the surface. It should be mentioned that, owing to compression of the soil, it was usually necessary to penetrate to a depth of about 11 ins. in order to fill the sampler. Thus the lowest of the six small samples would not in most cases have been disturbed by the rototiller.

These samples of soil of varying depth were examined for numbers of cysts and for the amounts of the chemical dressing which they had received. The cysts were separated and counted in the usual way and

TABLE I.

Number of Cysts per 100 gm. Soil.

Depth of Soil sample	Treatment					Average
	Control	$CaCN_2$ 40 c.p.a.	$CaCN_2$ 60 c.p.a.	$CaCN_2$ 80 c.p.a.	$(NH_4)_2SO_4$ 80 c.p.a. + $Ca(OH)_2$ 63 c.p.a.	
1st 1½″ (Top) ...	152	246	148	145	214	181
2nd 1½″ ...	156	160	151	188	167	164
3rd 1½″	171	159	168	169	217	177
4th 1½″	57	103	77	130	218	117
5th 1½″	27	35	36	55	100	51
6th 1½″ (Bottom)	9	9	5	5	4	6

the numbers obtained are given in Table I. These show that on this land the cysts were fairly evenly distributed amongst the soil to a depth of about 5 ins., but fell off in numbers at greater depths. As a measure of the amount of the chemical dressing which each sample contained, estimations of nitrogen on 0·5 gm. of soil were made with a micro-Kjeldahl apparatus. As a further indication of the amount of chemical present, *p*H measurements were carried out with a quinhydrone electrode. The results are shown in Tables II and III, and suggest that most of the chemical had in each case remained in the upper five or six inches of the soil. To test this, hatching experiments were devised.

TABLE II.

"Kjeldahl" Nitrogen (gm. per 100 gm. soil).

Depth of Soil Sample.	Treatment				
	Control	$CaCN_2$ 40 c.p.a.	$CaCN_2$ 60 c.p.a.	$CaCN_2$ 80 c.p.a.	$(NH_4)_2SO_4$ 80 c.p.a. $+Ca(OH)_2$ 63 c.p.a.
1st 1½" (Top) ...	0·22	0·32	0·36	0·34	0·34
2nd 1½"	0·24	0·29	0·32	0·34	0·35
3rd 1½"	0·24	0·28	0·28	0·32	0·32
4th 1½"	0·18	0·25	0·25	0·24	0·25
5th 1½"	0·16	0·18	0·20	0·24	0·21
6th 1½" (Bottom) ...	0·12	0·13	0·13	0·21	0·15

TABLE III.

pH (quinhydrone electrode.)

Depth of Soil Sample	Treatment				
	Control	$CaCN_2$ 40 c.p.a.	$CaCN_2$ 60 c.p.a.	$CaCN_2$ 80 c.p.a.	$(NH_4)_2SO_4$ 80 c.p.a. + $Ca(OH)_2$ 63 c.p.a.
1st 1½" (Top) ...	5·5	7·7	8·1	8·1	6·2
2nd 1½"	5·4	7·3	8·1	8·2	6·9
3rd 1½"	5·6	7·1	6·8	8·0	6·1
4th 1½"	5·1	5·7	5·4	5·9	5·3
5th 1½"	4·9	5·1	5·0	4·7	5·0
6th 1½" (Bottom) ...	4·8	5·0	4·7	4·5	4·9

The plots were left for eight weeks and samples were then taken. In this case the soil contained in the sampler was divided into two portions only, the upper and lower halves being withdrawn separately. The upper halves from each plot of the same treatment were mixed to form a composite sample, and similarly with the lower halves. The cysts were separated from the samples and placed in Petri dishes containing potato root excretion. These experiments were carried out in the

autumn and relatively few larvae were induced to hatch in any of the dishes. The results given in Table IV are, however, strictly comparable with each other. It is seen that the only samples in which the chemical

TABLE IV.
Number of Larvae which hatched from 100 Cysts

Depth of Soil Sample	Treatment				
	Control	$CaCN_2$ 40 c.p.a.	$CaCN_2$ 60 c.p.a.	$CaCN_2$ 80 c.p.a.	$(NH_4)_2SO_4$ 80 c.p.a. + $Ca(OH)_2$ 63 c.p.a.
Top 4½"	82	27	15	Nil	Nil
Bottom 4½"	94	146	121	181	29

appeared to have been lethal to the contents of all the cysts were the upper portions treated with calcium cyanamide and ammonium sulphate plus lime in the very heavy dressings of four tons per acre. Fewer larvae hatched from the upper samples treated with calcium cyanamide at the rates of 40 and 60 c.p.a. than from the control sample, but it is evident that the mixing of the chemical with the soil had not been thorough even in the surface layers. This follows from the results of numerous small scale laboratory experiments, which have shown that when an amount of calcium cyanamide corresponding to a dressing in the field of 40 c.p.a. to nine inches is mixed very carefully with infected soil, it is lethal to the eggs contained in all the cysts. It will be noticed that the largest numbers of larvae hatched from the cysts obtained from the lower halves of samples of soil which had received dressings of calcium cyanamide. This is in accordance with observations in laboratory experiments, that an amount of this chemical insufficient to be lethal to *H. schachtii* may, when mixed with infected soil, lead to a stimulation of the hatching power of the larvae.

The results given in this paper indicate that the field experiment in progress is not likely to produce beneficial effects extending over a period of several years, on any of the treated plots. The examination of methods of incorporating chemical dressings with infected land will receive further investigation.

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Pot Experiments on the Chemical Treatment of Soils Infected with the Potato and Oat Strains of *Heterodera schachtii*.

By Richard H. Hurst, B.Sc., Ph.D., F.I.C.

(*Senior Research Assistant for Biochemistry, Institute of Agricultural Parasitology, St. Albans.*)

I. Potato Strain of *Heterodera schachtii*.

(*a*) *Acidified Calcium Cyanamide.*

It was observed in laboratory experiments that the lethal action of dilute solutions of calcium cyanamide on the larvae of *Heterodera schachtii* was more rapid when these solutions were rendered slightly acid. This might be due to the rate of decomposition of calcium cyanamide in aqueous solutions of varying *p*H. The changes which cyanamide undergoes in acid and alkaline media have been described by Crowther & Richardson (1932). Solutions of commercial calcium cyanamide are moderately alkaline because of their lime content, and in this alkalinity any free cyanamide which is formed tends to polymerise to dicyanodiamide. Experiments with solutions of dicyanodiamide showed that this substance has very little toxic action on *H. schachtii* larvae. In acid solution, on the other hand, cyanamide is hydrolysed to urea. This substance becomes toxic when it decomposes to ammonia, but in the slightly acid solutions used the change would be a slow process. It is probable, therefore, that the degree of toxicity of calcium cyanamide solutions would be greatest where the cyanamide radicle could be retained unchanged for the longest period.

When calcium cyanamide is mixed with normal soils, the buffer action of the soil keeps the *p*H approximately neutral. Even acid soils, however, are for a period made slightly alkaline by heavy dressings of the chemical.

It was therefore decided to try the effect of acidifying soil infested with cysts of *H. schachtii*, immediately after applying a dressing of calcium cyanamide. The acid selected for these experiments was pyroligneous acid, which contains approximately 4 per cent. of acetic acid.

Acetic acid solutions were found to be more toxic to *H. schachtii* larvae than mineral acid solutions of the same *p*H. This appears to be connected with the ready vaporisation of acetic acid from its solution. A similar effect is shown by ammonia solutions, which are more toxic than caustic soda solutions of the same *p*H or even of the same normality.

Duplicate series of pots were prepared, containing 20 cwt. per acre of acetic acid (as pyroligneous acid), 10 cwt. per acre of acetic acid plus 10 cwt. per acre of calcium cyanamide, and 20 cwt. per acre of acetic acid plus 20 cwt. per acre of calcium cyanamide. Four control pots were also set up. Infected Lincolnshire soil was used and was kindly supplied by Mr. J. Wood, A.R.C.S., of the Kirton Agricultural Institute. The pots were kept moist for three weeks and a seed potato was then planted in each. Growth in all the treated pots was superior to that in the controls and there was conspicuous absence of weeds. The pots were allowed to remain until the end of the growing period and were then turned out. The cysts on the outer roots were counted and the tubers formed were weighed.

TABLE I.

Treatment	Average no. of cysts on outer roots	Average weight of tubers formed (grams)
Control	596	215
Acetic acid 20 cwt. per acre	71	294
Acetic acid 10 cwt. per acre + $CaCN_2$ 10 cwt. per acre	99	242
Acetic acid 20 cwt. per acre + $CaCN_2$ 20 cwt. per acre	55	572

The results are shown in Table I and it is seen that in all the treated pots the numbers of newly formed cysts were very much smaller than in the control pots. By comparison with results given later in this paper and shown in Table II, it is also seen that 10 cwt. of acetic acid plus 10 cwt. of cyanamide led to the formation of fewer new cysts than did 20 cwt.

of cyanamide alone. The results indicate that acetic acid, either in solution or adsorbed on charcoal, might be worthy of trial on a larger scale, but it is certain that very large quantities would be necessary.

(*b*) *Powdered* versus *Granular Calcium Cyanamide.*

The granular form of calcium cyanamide, which is supplied as a fertiliser, is very much more convenient to handle than the powdered form. It was thought improbable that it would be as effective against *H. schachtii* in infected soil, since it would not mix so intimately with soil as would the powdered form. On the other hand, the granular form might be expected to retain its toxicity for a longer period. For the comparison of the two forms, duplicate series of pots were prepared containing infected Lincolnshire soil mixed with dressings equivalent to 20, 30 and 40 cwt. per acre. The soil was kept moist for one month and potatoes were then planted. There were no obvious differences in the appearances of the plants of the two series. In both cases the plants in the soil which received 40 cwt. per acre of cyanamide were retarded in growth for several weeks, but grew rapidly after the first two months. At the end of the growing period the roots were examined for the presence of new cysts and the tubers formed were weighed. The results are given in Table II, and show that the powdered form of cyanamide was much more effective than the granular form in preventing the formation of new cysts. The weights of tubers produced were approximately the same in both series.

TABLE II.

Treatment		Average no. of cysts on outer roots.	Average weight of tubers formed (grams)
Control		596	215
Powdered calcium cyanamide	20 cwt. per acre	300	449
	30 cwt. per acre	2	557
	40 cwt. per acre	Nil	401
Granular calcium cyanamide	20 cwt. per acre	697	382
	30 cwt. per acre	158	590
	40 cwt. per acre	3·5	400

II. Oat Strain of *Heterodera schachtii*.

The presence of *H. schachtii* cysts on the roots of oat plants growing in Shropshire was reported by Edwards (1935). Mr. J. Hamer Davies was kind enough to supply us with soil from this district. The soil did not show a heavy infection, the cyst count being 33 per 50 grams of air-dried soil. The percentage fulness of the cysts was only 10·6. To confirm that none of the cysts were of the potato strain, potatoes were planted in two pots containing this soil. Examination of the roots showed that there was no infestation by *H. schachtii* larvae.

A duplicate series of pots was prepared containing dressings of calcium cyanamide equivalent to 1, 2, 3, 4 and 5 tons per acre. For comparison, a series was also prepared containing ammonium sulphate and lime with the same nitrogen and calcium contents as in the cyanamide series. Four control pots were set up. The soil was kept moist for five weeks and then three seeds of Black Tartarian oats were planted in each pot.

The control plants made poor growth, but otherwise did not show signs of oat-sickness. The number of new cysts formed on the roots was, on the average, only 5 per pot.

In the cyanamide series germination of the seeds was much retarded or failed altogether in the pots containing 2, 3, 4 and 5 tons per acre of the chemical. In the two pots containing 1 ton per acre, germination took place, but the first leaves formed were very unhealthy and showed whitening of the tips. In these two pots the rate of growth increased later and finally the plants were superior to the controls in size and yield of grain. The average number of new cysts formed on the roots was 15 per pot.

In the ammonium sulphate and lime series, germination proceeded normally with dressings corresponding with 1 and 2 tons per acre of the cyanamide series, but was much retarded with the heavier dressings. In no case, however, was whitening of the tips of the leaves observed. The dressing of 1 ton per acre gave a strong impetus to growth and the plants were much larger and healthier than any others in the experiment. An average of 3 new cysts per pot was obtained. In the pots which received 2 tons per acre or more, no cysts were formed on the roots.

When the above series of cyanamide pots was prepared, it was anticipated that germination might not take place in many cases if oat seeds were planted after a few weeks. Another exactly similar series was therefore prepared, along with two controls. The soil was kept

moist for five weeks to allow the cyanamide to exert its possible lethal action on the cyst contents. The pots were then heavily watered three or four times a week for a further two months. By this means, very little of the chemical or its decomposition products would remain to produce a fertiliser effect.

Oat seeds were planted and germination took place in all the pots. There was now no whitening of the tips of the leaves. The control plants were conspicuously poorer in size and appearance than any of the others. The plants in the pots which had received 1 ton per acre of cyanamide, although much better than the controls, were not equal to those in the pots which had received heavier dressings. There was, however, no difference in size or appearance of the plants following the dressings of 2, 3, 4 or 5 tons per acre of cyanamide. The number of cysts on the roots of the control plants averaged 5 per pot, whilst the number following 1 ton per acre of cyanamide was 2 per pot. In none of the other pots were cysts found on the roots. The similarity in appearance of the plants in these pots might, therefore, be explained by the absence of infestation by *H. schachtii* larvae, together with the leaching away of the chemical fertiliser as a result of the heavy watering. Since very few new cysts were formed on the roots of the control plants, this explanation would require the assumption that the oat plant is less able to withstand relatively slight eelworm infestations than is the potato plant.

It appears probable from these experiments that a suitably large dressing of calcium cyanamide, applied to oat-sick land in the autumn previous to growing a crop of oats, would result in an increased yield. It is, however, improbable that beneficial results of a permanent nature would be obtained, since present methods of cultivation on a field-scale do not secure a sufficiently intimate mixing of chemical with soil. For this reason, the chemical would not come into contact with many of the cysts in sufficient quantity to be lethal to the contained eggs.

Experiments on a field scale are being carried out to determine the efficiency of various methods of cultivation in securing even mixing of calcium cyanamide with soil infested with cysts of the potato strain of *H. schachtii*. Until such a method has been devised it is unlikely that freedom from eelworm attack will be obtained for more than one season by a single application of calcium cyanamide or any other lethal chemical.

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Experiments with Cysts of the Potato Eelworm (*Heterodera schachtii*) of Different Ages.

By Mary T. Franklin, B.Sc.

(*Attached to the Institute of Agricultural Parasitology, St. Albans, by the Agricultural Research Council.*)

One of the peculiarities of the eelworm *Heterodera schachtii* which makes it a very difficult parasite to control is the length of time it can survive in a dormant condition in the soil. In this state the embryonated eggs are protected by the resistant cyst wall, and, in the case of the potato strain of the eelworm, the larvae only hatch in large numbers in the presence of the host plant. On account of this property, land infected with the parasite remains infected even though the host plant is not grown. It is however well known that if "potato sick" land is rested from potatoes for a few years it is then sometimes possible to grow a potato crop giving a fairly good yield and showing little or no sign of sickness in the overground parts.* In these cases, however, it is often found that the roots of the plants are heavily infested with eelworm. These and other observations have led to suggestions that other factors may be associated with *H. schachtii* in giving rise to the condition known as "potato-sickness." Such factors may be due wholly or in part to soil conditions, climate, fungal attack or the time of attack by the eelworm. In some cases more than one factor may operate in addition to the nematode, and in different cases it is probable that different factors come into play.

It has been shown by Carroll & McMahon (1935) that the time of attack on the plant by the larvae influences the appearance or absence of symptoms of sickness. If the attack is delayed until a certain amount of root growth has been made the plant can withstand the parasite fairly well, but if the eelworm attacks from the beginning of growth the plant suffers badly. It was thought possible that cases of successful potato crops grown on heavily infested land which had not borne potatoes for several years might perhaps be explained by a delay in attack by the parasite owing to decreased vitality due to age. Delay might occur

* A case has recently been brought to the notice of the writer of a potato crop which failed badly although grown on land which had not grown potatoes for five years. Bad patches occurred in the field, and these were attributed to eelworm.

either in the response to the hatching stimulus given out by the potato roots, or in the journey of the larvae from the cyst to the host roots, or in both these processes.

An opportunity for investigating the behaviour of cysts of different ages arose when samples of soil containing cysts, but not having grown potatoes for different known periods of time, were made available. Three samples were very kindly collected in Yorkshire by Mr. A. E. Godfrey of West Butterwick ; these were from fields which had not grown potatoes for 1, 5 and 6 years respectively. Mr. J. Wood of the Kirton Agricultural Institute was also good enough to collect samples of infected soil which had not grown potatoes for 1, 2, 3, 4, 5, 7 and 8 years. Thanks are due to both these collectors, whose assistance is very much appreciated. Although the age of the youngest cysts in these samples is thus known, it is of course not possible to say what proportion of the cysts is of the minimum age.

It was decided to find out how rapidly, and to what extent, potato plants growing in contact with these cysts would become infected by the nematodes. The method adopted was to grow the plants in clean soil which had been artificially infected with the cysts of known minimum age, and, after certain periods of time, to remove the plants, stain the roots, and examine them for larvae. A comparison of the numbers of larvae found in the roots of plants grown in soil infected with the different cysts should thus give some indication of the rate of infection by larvae from the cysts of different ages.

Experimental Infection of Soil.

All the cysts were removed by flotation from the samples of soil. They were then mixed carefully in definite proportions with soil which had been sterilised six months previously, and the potato plants were grown in this soil. As it was essential that the soil should be equally infected in each case, it was of no use to add equal numbers of cysts of the different age groups to equal quantities of the sterilised soil, since the number of unhatched eggs contained in the cysts was certain to vary in the different groups. It was therefore decided to estimate the average number of full eggs in each cyst, and to infect the soil at the rate of 400 eggs per cc. of soil. This number was chosen as it represents an infection equivalent to about 2 full cysts per cc.of soil, which is generally considered to be a reasonably heavy infection.

Estimation of Number of Eggs per Cyst.

The method which was evolved for estimating the number of eggs in a cyst was based on the property possessed by calcium hypochlorite of dissolving cysts. It was shown by Smedley (1936) that this chemical will readily dissolve the cyst wall, setting free the enclosed eggs. Ten cysts from the sample to be examined were placed in 1 cc. of a 1% solution of calcium hypochlorite and kept under observation until the cyst wall had dissolved and the contained eggs had been released. The suspension was then made up to 10 cc. with water and shaken vigorously to separate the eggs. It was placed in a small Petri dish and immediately examined under the binocular microscope, and the full eggs were picked out with a pipette and counted. Many of the eggs contained a clearly visible embryo, but others had undifferentiated contents. All the eggs which had contents of any sort were counted, as changes might have been brought about by the action of the chemical causing the embryo to lose its definite form. Five counts were made for each sample of cysts, and the average number of eggs per cyst was calculated. The number of cysts required to infect the sterilised soil at the rate of 400 full eggs per cc. was then thoroughly mixed with the soil. (See Table I.)

TABLE I.

Counts of numbers of unhatched eggs in cysts of different ages.

Minimum Age of Cysts	Number of Full Eggs per 10 Cysts					Average per Cyst	No. of Cysts required per cc. of Soil
	1	2	3	4	5		
Yorks. Cysts.							
6 years	276	150	275	111	128	18·8	21
5 ,, ...	327	352	240	230	128	25·5	16
1 year	976	1,600	1,680	1,760	2,680	173·9	2
Lincs. Cysts.							
8 years	170	440	150	50	370	24	17
7 ,, ...	480	450	40	20	150	23	17
5 ,, ...	650	625	280	290	250	42	9
4 ,, ...	120	320	170	240	400	25	16
3 ,, ...	400	450	480	325	330	40	10
2 ,, ...	380	550	340	920	550	55	7
1 year	550	970	765	600	860	75	5

It will be noticed that in the Lincolnshire series the average number of eggs per cyst does not decrease regularly with the increasing age of the

samples of cysts. The cysts in the different samples, of course, belong to different populations and vary considerably in average size, and this might be thought to account for discrepancies in the average numbers of eggs per cyst in the different samples. Another factor which might influence the number of unhatched eggs remaining in the cysts is the cropping of the land since the last potato crop. It has been shown by Triffitt (1934) and by Franklin (1937) that in the presence of certain crops, notably grasses, a considerable amount of hatching of larvae from the cysts occurs. Some such factor as this may account for the irregular falling off in the numbers of full eggs in the cysts of the Lincolnshire samples.

It is realised that the counting of the unhatched eggs in a sample of cysts does not necessarily give a perfectly accurate idea of the infectability of the cysts in the sample. Some error probably arises from the fact that all unhatched eggs have to be counted, even though some would perhaps never have hatched owing to damage by soil organisms such as fungi or bacteria. As it is practically impossible to state definitely that a given egg would not have hatched, all which are not empty must be counted. It is probable that the error due to this factor is greater in older than in younger cysts, since there has been more opportunity for damage to the older cysts.

Growth of Potato Plants in Infected Soil.

Potato setts were cut from seed potatoes with a cork borer so that each had about the same amount of tuber attached. They were planted in damp sand, and after about a week's growth they were removed and planted in the artificially infected soil. Plants having as nearly as possible the same amount of root were chosen, the average length of the roots being 4½ to 5 ins. Some error arises here, since it is impossible to find a number of plants each with exactly the same amount of root. A plant with a large number of roots is supposedly more likely to become infected than one with few roots, since the larvae have more chance of finding the roots, and more root excretion is produced, which may stimulate the larvae to hatch in greater numbers.

As the quantity of soil which could be infected was rather restricted owing to the small numbers of cysts available, and the time for which the potato plants were to be grown in it was short, it was decided to grow the plants in threes in small plant pots about 3½ ins. in diameter

at the top. At the bottom of each pot was a small quantity of silver sand, then the infected soil in which were the roots of the three plants, and on top a covering layer of sand. There were three pots in the South Yorkshire series and seven in the Lincolnshire series, each pot having soil infected with cysts of a different minimum age, and within each series, containing the same volume of soil infected with approximately the same numbers of unhatched eggs per cc.

Three days after the setts had been planted in the pots one plant was removed from each pot and the roots were fixed and stained. A second plant was removed from each pot five days after planting, and the third two days later. Control plants were grown in sterilised soil without the addition of cysts and treated in exactly the same manner.

Staining of Roots and Counting of Larvae.

Two methods of staining were used. The first was by means of Flemming's chromo-aceto-osmic acid. The roots were placed in this and left for three or four hours; they were then dehydrated and cleared in beechwood creosote. On examination under the binocular microscope the larvae were seen as black objects in the cleared roots. After a time this method was replaced by a quicker one. The roots, on removal from the soil, were put straight into boiling lactophenol coloured with acid fuchsin as described by Putnam & Chapman (1935 p. 640). After a minute or two they were removed and placed for clearing in colourless lactophenol or creosote. The larvae showed up well, and where there was any doubt as to their identity they were removed from the roots and examined under the compound microscope, the internal structure being clearly distinguishable. This is an advantage over the Flemming method where the structure was usually obscured by the density of the stain. The numbers of larvae found in the roots in the two series are shown in Table II (on page 72).

The error due to differences in amount of root in the different plants is most pronounced in those plants which were allowed to grow for seven days, as during this time some plants made appreciably more root growth than others.

It appears from these results that even after so short a period as three days the roots of potatoes growing in soil infected with *H. schachtii* may become invaded by larvae of the parasite. In the case of the cysts from

Yorkshire only the one-year-old cysts had infected the host in this period. The five- and six-year-old cysts had infected the host at the end of five days, though the invasion by the six-year-old cysts was only slight. In this series the five-year-old cysts gave rise to a heavier infection than the one-year-old ones, but the six-year-old cysts caused considerably less infection than the latter.

TABLE II.

Numbers of larvae found in roots of plants growing with cysts of different ages.

Age of Cysts (minimum)	Numbers of Larvae Found in Roots.		
	3 Days	5 Days	7 Days
Yorks. Cysts.			
6 years	0	5	76
5 ,, ...	0	164	783
1 year	4	42	164
Control	0	0	0
Lincs. Cysts.			
8 years	1	219	1,500 (approx.)
7 ,, ...	7	185	2,200 (,,)
5 ,, ...	3	36	732
4 ,, ...	0	241	837
3 ,, ...	0	157	1,430
2 ,, ...	2 (roots poor)	154	2,200 (approx : very many roots)
1 year	70	874	1,377
Control	0	0	0

Invasion of the potato roots by larvae from the Lincolnshire cysts also took place very rapidly, even in the case of some of the older cysts, but the youngest cysts had produced a much heavier infection both after three and five days than any of the others. At the end of a week all the cysts had given rise to a surprisingly heavy invasion of the host roots, but the youngest ones showed no appreciable difference from the other groups. Apart from the greater infectiveness shown by the youngest cysts in the first five days there is very little difference to be observed between the behaviour of the cysts of the various groups. The five-year-old group showed a much slighter infection than older and younger cysts both after five and seven days, but apart from this, all cysts above one year old seem to be fairly equal in the rate at which they cause invasion of the host plant.

Position of Larvae in the Roots.

During the counting of the larvae in the potato roots it was noticed that the distance of the nematodes from the tip of the roots was variable. It was thought that some indication of the time which had elapsed since the entry of a larva into a root should be given by its distance from the root-tip, since the point of entry is usually immediately behind the tip. The distance of the larva from the tip depends largely on the growth of the root after the entry of the parasite ; this should be similar in the different plants in the experiment, thus the distance of the larvae from the root-tips should give some idea of when the infection took place. Accordingly, measurements were made of the distance from the root-tip of the first 25, or in some cases the first 50, larvae met with during the counting of the larvae in the roots of the plants which had been allowed to grow for seven days in the soil infected with Lincolnshire cysts. The distance measured was that between the end of the larva nearest the tip of the root and the tip itself.

The results of these measurements (see Table III) bear out the indications obtained from counts of larvae in the roots, and tend to show that one-year-old cysts produced the first invasion of the roots, but that there was not much difference in the rates of invasion by the larvae of the older groups.

TABLE III.

Position of larvae in host roots after seven days.

(Lincolnshire cysts.)

Age of Cysts.	No. of Measurements	Percentage over 10mm. from tip	Percentage 5–10mm. from tip	Percentage 1–5 mm. from tip	Percentage 1mm. and less from tip	Average distance from tip
8 years ...	50	4	12	60	24	2·8mm.
7 „ ...	50	14	18	46	22	4·6 „
5 „ ...	25	7·4	33·2	59·2	0	4·6 „
4 „ ...	25	0	0	88	12	1·7 „
3 „ ...	25	0	8	64	28	2·3 „
2 „ ...	50	0	16	68	16	2·8 „
1 year ...	50	20	20	50	10	5·8 „

After seven days it appears that the greatest numbers of larvae at a distance of over 10 mm. from the root-tips are in those roots growing in the soil infected with one-year-old cysts, and that the larvae from these

cysts are on the average at a greater distance from the tips than the larvae from the older cysts. Apart from these facts the measurements obtained are so erratic as to indicate that a much greater number of measurements might profitably have been made.

Rate of Hatching *in vitro* of Larvae from Cysts of Different Ages.

Hatching experiments with cysts from the samples used in these investigations were next undertaken, as it was thought that they might throw some light on the behaviour of the different cysts. There were not sufficient cysts available to make direct comparisons of the numbers of larvae liberated from the various samples, but frequent counts of larvae were made so that the period at which hatching was taking place at the greatest rate could be compared in the different cases. It was thought possible that the time taken for the rate of hatching to reach the maximum might vary in the different samples of cysts which were being used.

The cysts on which the hatching observations were to be made were placed in Petri dishes in a small quantity of water which had been allowed to percolate through a pot of soil in which a potato plant was growing, and had then been filtered. At intervals of several days the larvae were counted and removed from the dishes. Fresh leachings from soil in which a potato was growing were substituted for the old solution once or twice a week.

Of the Yorkshire series the one- and five-year-old cysts only were available for comparison, the oldest batch having all been used in previous experiments. It was found that the greatest number of larvae emerged from each batch during the second week. In the third week the number of larvae counted from the older cysts fell almost to the same number as emerged during the first week. The fall in numbers occurring in the one-year-old cysts in the third week was, however, comparatively slight, nearly twice as many larvae hatching as hatched in the first week. The numbers of larvae hatching in the fourth week fell again considerably in both cases. Similar results were obtained with the Lincolnshire cysts, only the youngest keeping up a high rate of hatching after the second week. The four-year-old cysts behaved more like the one-year-old ones than the others, but were peculiar in having an exceptionally low rate of hatching in the first week. The details of the results are given in

Table IV. In the results with the Lincolnshire cysts the figures for the one-year-old cysts are the average of three separate batches, while two batches were set up and are averaged in the case of the four-, five- and seven-year-old cysts; only one batch was available of three- and eight-year-old cysts.

TABLE IV.

Hatching of larvae from cysts of different ages.

Yorkshire Cysts. Larvae hatching from 100 cysts.

Age of Cysts.	9 *days*	10–13*th day*	14–16*th day*	17–28*th day*
1 year	688	2,185	1,762	151
5 years	548	816	543	16

Lincolnshire Cysts. Larvae hatching from 54 cysts.

Age of Cysts.	9 *days*	10–13*th day*	14–20*th day*	21–28*th day*
1 year	621	1,052	1,020	147
3 years	684	1,015	497	29
4 „	75	1,244	1,092	143
5 „	970	1,249	602	18
7 „	370	686	468	14
8 „	350	1,209	357	18

It appears that over a period of about a month the maximum rate of hatching from these cysts occurred at approximately the same time after stimulation with potato root excretions, that is to say, during the second week. The liberation of larvae, however, fell off during the third week in the case of the older cysts, but the number freed from the one-year-old cysts remained fairly high until the fourth week.

Conclusions.

From the investigations carried out with cysts of different ages it appears that on the whole one-year-old cysts are likely to do more harm to a potato crop than older cysts. Invasion of the roots by larvae hatching from the former appears to be more rapid during the first few days and to occur in greater numbers than by larvae hatching from older cysts. The higher rate of hatching would appear to be sustained for a longer period by the younger cysts than by those which have lain dormant in the soil for two or more years. That the time of attack is an important

factor in the pathology of potato sickness has been shown by Carroll & McMahon, and it may be that the delay in attack in the case of the older cysts, though seemingly slight in the pot experiments described above, is nevertheless sometimes sufficient, if other conditions are favourable, to enable the plant to stand up to the attack. In these experiments there seemed to be little difference in the intensity of infection by the larvae from the different cysts after a week had elapsed, but the hatching tests indicated that the rate of hatching was sustained at a high level for a longer period by the youngest cysts than by any of the older ones. These two factors, though not apparently very striking, might be sufficient to upset the balance in the economy of the plant where conditions were not very favourable for it, and cause the development of symptoms of potato sickness.

The practical indications of these experiments seem to be that, as far as the eelworm factor is concerned, although potato sickness may in some cases not show itself if the land is rested from potatoes for at least one season, even if as long as eight years is allowed to elapse between two potato crops there may be a severe attack, and the infection in the soil may be thereby increased by the addition of new cysts.

ACKNOWLEDGMENTS.

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Some Nematode Parasites found in Chinese Water Deer (*Hydropotes inermis*), with a Description of *Trichostrongylus cervarius* n. sp.

By J. W. G. Leiper, M.R.C.V.S. and Phyllis A. Clapham, Ph.D.

(*Research Assistants, Institute of Agricultural Parasitology, St. Albans.*)

Through the courtesy of Capt. Beal of the Zoological Society of London and Mr. A. D. Middleton of the Bureau of Animal Population, Oxford, we had the opportunity of examining the viscera of some Chinese Water Deer which formed part of the stock of the Zoological Society of London and which were being kept at Whipsnade Park in Bedfordshire. Owing to excessive increase of the herd, it was decided by the authorities to reduce the numbers in the Spring of the year 1937, and so we were provided with 24 specimens. The animals were all healthy and were shot without discrimination as to age or sex though for the laboratory examination they were divided into two groups—those over one year of age, of which there were 14, and those under one year, of which there were 11 specimens.

These deer were originally introduced 4 years earlier but probably all the animals examined were bred at Whipsnade. As they had been grazed on land part of which for many years had carried cattle, the rest being arable, the parasites which were found were not necessarily those which would be found in their natural habitat in China. It will be shown later that most of the parasites found, with the exception of the new species of *Trichostrongylus*, are all commonly found in this district and are indeed widely distributed throughout the world; though *Osterlagia lyrata* seems to be limited to Europe, Africa and America, and *O. grühneri* to Asia and Europe.

Not all the livers, lungs and rumina were examined for helminths as a complete examination of 6 showed that no parasites were present. The alimentary canal was examined in 4 sections, *viz.*, abomasum, duodenum, ileum and jejunum, and the large intestine. The contents were washed out and sieved and the parasites picked out by hand. The following parasites were found :—*Bunostomum trigonocephalum, Capillaria longipes, Chabertia ovina, Haemonchus contortus, Nematodirus fillicollis,*

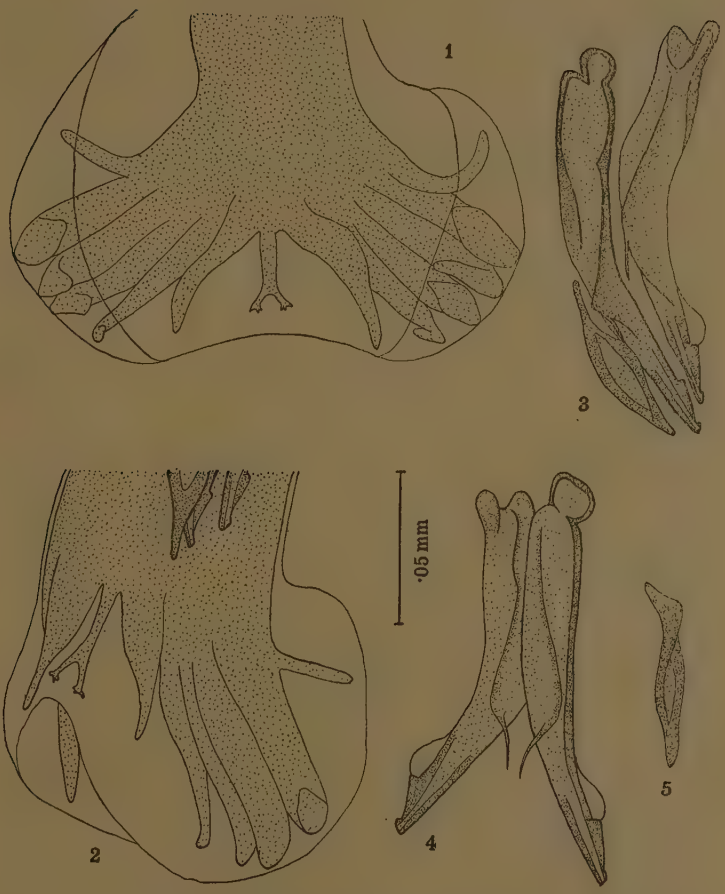
Ostertagia lyrata, *O. grühneri*, *Strongyloides* sp., *Trichostrongylus axei*, *T. retortaeformis*, *T. colubriformis*, *T. vitrinus* and *T. cervarius*.

While the same species infected both young and old animals, it was noticeable that on the whole, *O. lyrata* and *O. grühneri* infested older animals more frequently than the young. The infections were also heavier. *Trichuris ovis* occurred in only one of the old hosts but was present in 7 of the young ones.

The worm burden varied from as low as a total of 28 parasites to nearly 5,000. None of the animals however could be considered to have a pathological infection.

Although 14 species have been recovered from these Water Deer, all of which are new records, some of them were very rare. *Bunostomum trigonocephalum* occurred 6 times, one specimen being recovered from the small intestine of each animal. *Haemonchus contortus* occurred in the duodenum of an old animal, *Chabertia ovina* was present in two animals, one young and the other old, but it was, however, present in fairly large numbers in the large intestine. *Nematodirus fillicollis* was recovered from the small intestine of 6 animals, while *Trichostrongylus retortaeformis*, also in the small intestine, occurred in two only. *Strongyloides* sp. was an infrequent parasite, occurring in the duodenum and small intestine of 5 of the young ones in small numbers. Of the other helminths, *Trichostrongylus* and *Capillaria* were the most abundant genera, occurring in every animal examined, while *Ostertagia* was only absent from a few, being present in the abomasum.

Some little difficulty was experienced in the diagnosis of the *Ostertagia* specimens. We were clearly dealing with two distinct species, which we have identified as *O. lyrata* and *O. grühneri*, though there are some small points of difference. To consider first the specimens which we consider to be *O. lyrata*. They agree in most respects with those described by Sjøberg in 1926, but the median process of the spicules shows, in certain positions, a termination shaped like a ploughshare, such as has been figured for *O. arctica*. This latter species corresponds to a large extent, however, with *O. lyrata* and except for the shape of the gubernaculum might even be identical. Our *O. lyrata* has also a longer dorsal ray in the bursa than has generally been figured, but in spite of these two points we feel that they must be considered as belonging to this species rather than to some other. Such differences are of minor importance and not of sufficient value for the creation of another species, as would be



Trichostrongylus cervarius n. sp.

Fig. 1. Bursa, artificially spread out to show the arrangement of the rays—ventral view.

Fig. 2. Bursa, in its natural position—latero-dorsal view.

Fig. 3. Spicules—lateral view.

Fig. 4. Spicules—ventral view.

Fig. 5. Gubernaculum—latero-ventral view.

necessary. The males show some resemblance to *O. occidentalis*, *O. skrjabini* and *O. trifida*, three species which are, however, closely related and which Travassos (1937) seems to think may even be identical. Our specimens can, however, be differentiated from these by their possession of much slenderer spicules. This species was recovered from 14 of these slaughtered animals: it has previously been recorded from the ox *Bos taurus*, sheep *Ovis aries*, the American Fallow Deer *Odocoileus virginianus* and the Chamois *Rupicapra rupicapra* and is fairly widespread in Europe, Africa and America.

O. grühneri, previously recorded as a parasite of Reindeer *Rangifer tarandus* in Asia and Europe, was discovered in 16 of these Deer, being equally abundant in the young and the old.

We believe that these two species of *Ostertagia* were imported with the original stock, as they have not been recorded from anywhere in England before.

Trichuris ovis was one of the larger helminths obtained from the large intestines of these animals. It was a parasite mainly of the young stock, though an old deer had 21 specimens. It was a light infestation throughout for except for one animal which had 24 worms, the numbers were well under 20.

The genus *Capillaria* was represented by the species *C. longipes* and occurred in the small and large intestines of every animal examined, the infections being much heavier in the young. Several of them carried over 200 worms, while the old ones had not more than 35.

Five species of the genus *Trichostrongylus* were present, in the small intestine only, except for *T. axei* which was most abundant in the abomasum. Of these the presence of *T. retortaeformis* is interesting in that it is normally a parasite of rabbits and hares and until recently (Leiper, 1937) had not been recorded as a natural parasite of Ungulata. Two of these Deer, both young animals carried a light infection—under 100 worms of this species. On the whole the young animals carried heavier infections of *Trichostrongylus* than the old ones. *T. axei* occurred in practically all, in the abomasum; *T. colubriformis* also occurred in all the young ones and about half the old ones: *T. vitrinus* occurred in the same number of animals but the infections were lighter. The last species to be noted is *T. cervarius*, a species which was recovered in large numbers from all the small intestines examined, and which has not hitherto been described.

Trichostrongylus cervarius, n. sp.

The new Trichostrongylid obtained from the duodenum is a typical member of the genus. We were unable to identify the females from those of other species, but the male measures from 4·4 mm. to 6·2 mm. in length. The bursa is well developed, but the dorsal lobe is completely absent. The lateral lobes tend to be curled inwards and the bursa is found typically in a half closed position (Fig. 2). The dorsal ray measures 31 μ to 37 μ in length : at its posterior third it divides, each branch remaining the same thickness for about 10 μ. At this point a further division takes place and the inner branch bifurcates almost immediately so that there are finally three points on each side. There is a very gradual tapering of the main stem from its root to the point at which it first bifurcates. The externo-dorsal rays are sturdy, arising at an angle of 60° from the dorsal ray. The postero-lateral and medio-lateral rays are of approximately the same size and run parallel for some distance, but finally become divergent. The antero-lateral and latero-ventral rays are the longest and biggest rays and run close to one another and to the medio-lateral. The ventro-ventral ray is small and very much divergent from the latero-ventral.

The spicules (Figs. 3 and 4) are strongly chitinised, sub-equal and appear dark brown in colour. Their length varies from 121 μ to 136 μ. Each possesses a well defined process terminally shaped something like a ploughshare and measuring 21 μ in length. They are very conspicuous in lateral view. About 7 μ from the tip there is a sudden widening of this process at right angles to the edge and the actual tip is blunt. The body of the spicule is considerably ridged, one ridge being particularly prominent, being roughly triangular in shape. One edge is continued into a very fine process. A transparent membrane, rounded in outline, springs from the edge of the spicule and is connected with the ploughshare-like process.

This spicular shape is absolutely diagnostic of this species, and though it is similar to *T. colubriformis*, differs in quite distinct fashion.

The gubernaculum (Fig. 5) measures 60 μ in length and broadens in the middle, corresponding in ventral view to that of *T. capricola*. Latero-ventrally, however, it is seen that the anterior end is thickened and drawn out to form an oblique process.

The differences from *T. colubriformis* and *T. capricola* which we have observed are so definite and constant in their appearance that we must

constitute a new species for the reception of these specimens. As the host is a member of the Cervidae, we propose the specific name *cervarius*.

It is highly probable that this species may have been a natural parasite of water deer in their original home in China, and was brought over when the original stock was imported.

Most of the parasites recovered from these animals are common inhabitants of domestic ruminants in England, and it is impossible to say whether or no they are normal parasites of this host.

Summary.

(1). The helminthic fauna of a number of Water Deer, *Hydropotes inermis* from Whipsnade Park has been examined.

(2). Fourteen species of Nematodes were recovered, all of which were new records for this host.

(3). *Ostertagia lyrata* and *O. grühneri* are recorded for the first time in England.

(4). *Trichostrongylus cervarius* n. sp is described.

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Eosinophilia and the Differential Blood Count in Trichinosis of the Rat.

By VERNON D. VAN SOMEREN, B.Sc.

(*Research Student, Department of Parasitology, London School of Hygiene and Tropical Medicine.*)

INTRODUCTION.

A MARKED degree of eosinophilia is a fairly constant symptom of uncomplicated trichinosis in mammals, and taken in conjunction with other findings is of considerable diagnostic importance. While there are many reports of eosinophilia in clinical cases of trichinosis in man, the blood changes in trichinosed experimental animals have not been so fully studied.

Maass (1933) describes the blood changes in trichinosed swine, and claims that the degree of eosinophilia is in direct proportion to the initial dose of infective larvae; it occurs early and regularly, and increases steadily to a maximum 20–24 days after infection, accompanied by a leucocytosis also in proportion to the number of worms ingested. The eosinophilia decreases steadily to the normal percentage after the maximum has been reached, the latter not being maintained for more than one or two days. Wantland (1937) has studied the blood of normal and trichinosed white rabbits and shows that variations occur in all the factors studied in normal rabbits, but in rabbits infected with 5,000–10,000 larvae, a leucocytosis develops from the second week after infection, and eosinophilia (25–79%) during the third week after infection. Those animals showing the severest symptoms also showed a more marked eosinophilia, but Wantland concludes that the percentage of eosinophiles is not an index of the severity of the infection. The eosinophilia may persist at a high level for several months after infection as in man (Theiler, Augustine & Spink, 1935). No aneosinophilia was observed, though this is stated to occur in very severe and atypical cases (Conner, 1929); Spink (1934) has shown that the eosinophilia in trichinosed guinea-pigs may be reduced by complicating infections with *B. tuberculosis*, *Staphylococcus aureus*, and *Trypanosoma equiperdum*. McCoy (1932) was unable to demonstrate marked eosinophilia in trichinosed monkeys.

In man, the most complete study of eosinophilia of trichinosis still remains that of Brown (1898), although this author's suggestions as to the origin of the eosinophiles are incorrect.

No work has been done on the blood count changes in the rat, although it is commonly used for work on experimental trichinosis ; and moreover no account exists of the changes immediately following infection in any animal except the pig. These observations may help to fill these deficiencies.

Materials and Methods.

A series of 12 adolescent and adult white rats were infected with varying doses of encysted larvae ; 2 with 15 larvae, 2 with 55–60 larvae, 6 with 400–450 larvae, and 2 with 600 larvae. Definite numbers of predigested larvae were not used, because the cysts contents released in the stomach may be responsible for some of the early blood changes. In addition 12 other rats infected with about 500 larvae were examined at less frequent intervals.

A differential blood count was carried out every 2 days on all the rats for a week to a fortnight before infection, and also on a normal uninfected control rat kept under the same conditions, on each occasion during the observations. After infection the blood was examined every day for a week, then every two days for a fortnight, and thereafter at intervals of a week or more.

Blood is readily obtained by puncturing the tip of the tail, and was taken at the same time each day for each rat before feeding. The smears were stained with Giemsa, and in addition a direct count of the eosinophiles was made on a " thin thick-drop " dehaemoglobinised and stained for 10 minutes in weak neutral Giemsa (20 drops stain in 100 c.c. neutral distilled water). With correct staining and preparation it is possible to make a simple differential count of neutrophiles, eosinophiles, basophiles and lymphocytes, but not monocytes, on such a " thin thick-drop," overcoming the difficulty of differential distribution of leucocytes in a smear and rendering the whole count easier to perform. Such counts should be checked against a smear count, but will seldom be found to differ.

Total leucocyte counts were not carried out, hence this study deals only with the relative variations in leucocyte ratios.

THE NORMAL DIFFERENTIAL BLOOD COUNT OF THE RAT.

The only figures available for the normal differential blood count of the white laboratory rat are those of Donaldson (1915) who gives the following percentage ranges :—

Polymorphs	42·4–71%
Small lymphocytes ...	20–51·9%
Large lymphocytes ...	4–9·1% (Monocytes ?)
Eosinophiles	0–3%
Basophiles	0–·85%

The figures obtained by the writer from the uninfected rats and control over a period of four months are not in accord with those of Donaldson with regard to the percentages of neutrophiles and small lymphocytes. While marked variations occur from day to day in the same animal, the percentages of neutrophiles in all the rats were always lower and the percentages of small lymphocytes higher than those given by Donaldson. Ranges in the control rat, a typical normal count, were as follows ;

Neutrophiles ...	20·5–40%	Av. 31·1%
Small lymphocytes	57–78%	Av. 63·2%
Monocytes	2– 6%	Av. 3·4%
Eosinophiles ...	0– 4%	Av. 2·5%
Basophiles	0– 1%	Av. 0·3%

Thus it will be seen that neutrophiles and lymphocytes are in inverse proportion to the corresponding percentages in man—a point of some interest, since any stimulation for the production of granulocytes only has at once a more noticeable effect in the rat.* Neutrophile percentages of 60% or over consistently maintained in any rat before infection with Trichinella were an unfavourable sign, usually indicating a pre-existing bacterial infection from which the rat subsequently died. Such rats were not used in these experiments, since the count was already abnormal. No significant differences were observed in the counts of adolescent and adult rats.

It is evident from Maass's (1933) figures that the pig compares with the rat in this respect, neutrophiles and lymphocytes being in inverse

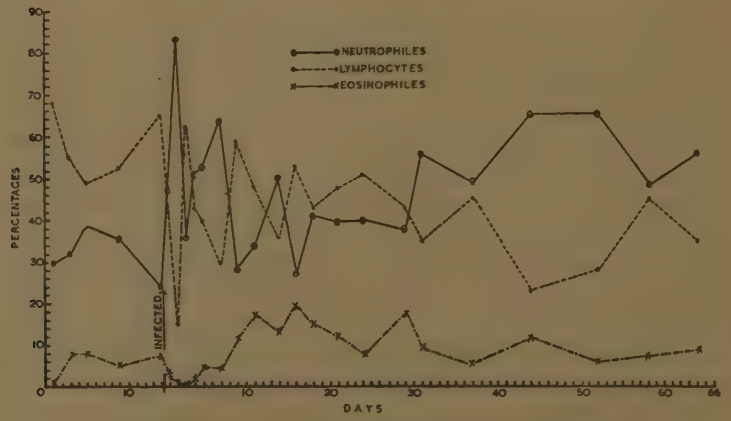
*Gulland and Goodall (*The Blood* 1925) give the following figures for the differential blood count of the rat; polymorphs 28%, lymphocytes 68%, eosinophiles 3%, and basophiles 1%. These figures are in agreement with the present findings, and these authors also comment on the number of neutrophiles and eosinophiles which show ring-shaped nuclei, an observation confirmed in the present study. These forms seem to be characteristic for the rat, and since they are especially numerous in the bone-marrow, they are probably young forms.

proportion to those of man, but it should be noted that in the pig and the rat, the fluctuations from day to day are much more marked than is normally the case in man. From figures quoted by Wantland (1937) the same conditions are apparent in the rabbit.

The polynuclear count (Cooke) of the normal rat also differs from that of man, though the greatest percentage of neutrophiles is also in class 3; the average count is as follows;

Class	1	2	3	4	5
	8	18	48	20	6

About half the number of rats examined before infection showed a fluctuating low-grade eosinophilia of about 4–9%. No obvious cause for this could be discovered, but since a number of these rats were infected with the scab mite, *Notoedras muris*, especially on the tail, this skin infection may be a possible explanation of this low-grade eosinophilia. The blood counts of these rats was otherwise normal, and this pre-existing low eosinophilia served to emphasise the changes occurring after infection.



Leucocyte ratios in Rat infected with 600 Trichinella larvae.

THE DIFFERENTIAL BLOOD COUNT OF TRICHINOSED RATS.

Fig. 1 is a graph of the typical leucocyte ratios in an infected rat (dose 600 larvae). This rat was one showing a fluctuating low eosinophilia before infection, but neutrophiles and lymphocytes were within normal limits; the counts obtained after infection were typical for all the rats infected with the greater doses.

Within 24 hours of infection a change is noticeable, the neutrophile percentage rising and the lymphocyte percentage falling, and this swing is at its maximum 2 days after infection in the 600 larvae group and in 4 days in the 400 larvae group, but was not noticed in the rats given the smaller doses.

	Rat I (Inf. with 600 larvae.)		Rat II (Inf. with 400 larvae.)	
	Neutrophiles	Lymphocytes	Neutrophiles	Lymphocytes
Before Infection % Range ...	21·5–39	50–67	33–38	57–64
Before Infection % Average ...	25·3	60·6	35·5	60·5
After Infection % Range ...	32–78	19–63	27–70	29–64
After Infection % Average ...	48	45	51	44
After Infection % Average excluding neutro. max. and lymph. min.	44	46	50	49
Max. Eosinophilia % ...	13		10	

Average Polynuclear count (Cooke) Before Infection.	Rat I					Rat II				
	Class 1	2	3	4	5	Class 1	2	3	4	5
	% 9	18	45	20	8	% 7	18	51	20	4

Days after Infection.	% in each class.					% in each class.				
1	10	14	38	24	14	10	30	48	8	4
2	12	24	42	18	4	—	—	—	—	—
4	—	—	—	—	—	14	28	40	12	6
7	16	26	42	16	0	—	—	—	—	—
21	—	—	—	—	—	12	24	52	12	0
30	6	32	48	12	2	—	—	—	—	—
75	10	28	46	16	0	—	—	—	—	—

A sum total of Classes 1 and 2 over 35 indicates a left shift.

In the two rats given 600 larvae each, neutrophiles rose to 83% and 79% and lymphocytes fell to 15% and 19% respectively, while in the rats given 400 larvae each the neutrophile maxima were 53–78% and the lymphocyte minima 47–22%. Thereafter the fluctuations became less, though the neutrophile percentages remained consistently higher than average and the lymphocyte percentages lower than average (Table). Whether these changes are absolute is uncertain without total leucocyte counts, but smear comparisons before and after infection suggest that they are absolute as well as relative, though leucocytosis has been stated not to develop until a week or more after infection. This swing

actually is apparent within 5½ hours of infection, but these figures are not included since digestion also produces a physiological leucocytosis which may complicate the picture.

Cooke polynuclear counts show that this reversal in proportions of neutrophiles and lymphocytes is accompanied by a moderate left shift, which is apparent within 1–2 days of the infection and continues for at least two months after infection. (Table.)

The percentages of monocytes and basophiles throughout the infection are not significantly altered, as has been noticed by other workers.

Immediately after infection the percentage of eosinophiles drops, this being especially marked in those rats showing an existing low-grade eosinophilia, and there supervenes a period of 1–5 days with eosinopenia or aneosinophilia, eosinophiles being absent or minimum in the peripheral blood in 2–4 days. Thereafter the percentage rises and attains its maximum in 7–16 days, this varying with the individual animals and not the infective doses. Rats given 600 larvae showed maxima of 20% and 13% on the 16th and 11th day respectively, while rats given 400 larvae each showed maxima of 6–10% in 7–14 days.

Rats given 15 larvae showed no period of eosinopenia, no neutrophilia, and a slight eosinophilia up to 7%. Rats given 60 larvae also showed no eosinopenia, or neutrophilia, and a maximum eosinophilia of 11–14% in 22 days. Since however neither of the latter two groups of rats showed living encysted larvae on autopsy, the value of these results is doubtful.

In all cases however, the eosinophilia is a fluctuating quantity and there may be two or more rises with intervening periods of low eosinophilia, but in the majority of rats a low-grade eosinophilia of 8–9% persisted for 2–4 months. In some rats examined, the aneosinophilia persisted, and in these cases death invariably supervened within a fortnight.

Discussion.

No record exists of the blood changes immediately following infection in man, owing to the difficulty of diagnosing the disease sufficiently early, nor are Wantland's (*loc. cit.*) observations sufficiently frequent for the period immediately following infection in rabbits. Maass (*loc. cit.*) makes no comment on any such neutrophile-lymphocyte swing in the pig early in infection, nor can any be made out from his figures since ranges only are given. It is significant however that the maximal neutrophile percentages for infected pigs are significantly higher than the maximum percentage of the infected control, and conversely the minimal

lymphocyte percentages are significantly lower than in the control. No left shift is evident from these figures, but possibly the toxaemia is not sufficiently profound to cause a marked alteration in the Schilling counts carried out by this author.

Brown's cases in man (*loc. cit.*) were diagnosed when the disease was well established, but the figures clearly show the leucocytosis with concurrent neutrophilia and eosinophilia typical of trichinosis, and it is significant that in the earlier stages the lymphocytes are relatively and absolutely *decreased*, though later they may be absolutely increased. Brown lays emphasis on the inverse proportions of the eosinophiles and neutrophiles, but it should be noted that these cases were very severe, with eosinophilia up to 64%, unlike the experimental cases studied; neutrophiles being more abundant in human blood than other types of leucocytes, it is natural to expect they would be reduced relatively with any marked increase of the less abundant types. During the phases of very high eosinophilia in these cases however, it is evident that neutrophiles were in fact absolutely decreased.

Eosinophilia in the pig apparently does not fluctuate so markedly as in the rat with comparable doses, and it is a curious fact that there is no period of eosinopenia or aneosinophilia immediately following infection, the percentage of eosinophiles in the peripheral blood rising the day after infection. Such a period of eosinopenia and aneosinophilia as found in the rat would naturally be expected following release and fixation of the larvae, and in fact sections of the intestine during this early period clearly show a local infiltration of eosinophiles into the sub-mucosa of the villi, these being recruited from those already present in the peripheral blood and the earliest formed in the bone marrow, thus giving the apparent reduction in the peripheral blood; Weinberg and Séguin (1914) have shown there can be no local eosinophilia unless eosinophiles are already present in the circulating blood. It is a significant fact moreover, that a generalised eosinophilia does not become apparent in most trichinosed animals until larviposition commences, and the larvae become dispersed through the body; and all the eosinophiles in the peripheral blood are mature forms, no myelocytes being included.†

†Wells (*Chemical Pathology*, 1925, p. 123) has suggested that the eosinophilotactic substances are elaborated only by the free larvae, or by the degenerated muscles in which they come to lie, and that the cyst contents are not active in this respect since eosinophilia is not evident until several days after infection. The setting up of an almost immediate local eosinophilia in the intestine, as described above would, however, modify this view.

It is possible also that part of the apparent reduction of lymphocytes in this early phase may be due to a local infiltration of the villi, since the villi of an infected intestine appear early congested and densely packed with cells, these however not including any neutrophiles.

Correlating these facts, it appears that a moderate Trichinella infection rapidly acts as a stimulus to the granulocytogenic tissue of the bone marrow, and causes proliferation not only of eosinophiles but also of neutrophiles, this being particularly marked in mammals such as the rat with a normal low percentage of the latter cells. Weinberg and Séguin (*loc. cit.*) have noted that eosinophilotactic substances may provoke an influx of neutrophiles and are not exclusively stimulating to the eosinophile. The early left shift in the polynuclear count clearly shows bone marrow stimulation, and not merely an influx of reserve neutrophiles from the spleen and other organs.

This leucocytosis persists for a considerable time, but in very severe cases such as those of Brown, there may later be stimulation of the lymphocytogenic tissue, and emphasis on the production of eosinophiles only. The degree of eosinophilia appears to be only generally related to the severity of the infection, different species and individual animals of the same species differing quite markedly in the degree of eosinophilia produced by the same infective doses (*e.g.*, 600 larvae in the rat produced an eosinophilia similar to an infection with 1,000 larvae in the pig). The persistence of aneosinophilia in the rat, as in other animals, is an unfavourable prognostic sign.

Whether these early changes would be so marked in infections with predigested larvae, where no release of cyst contents in the stomach occurs, is a matter for further experiment. The early diagnosis of trichinosis is of great importance from the point of view of instituting immediate treatment directed against the immature forms in the intestine, but even the Bachman intradermal skin test, which may be relied upon where clinical symptoms are indefinite, is not positive until a week or more after infection in laboratory animals, and about 16 days in man (Augustine and Theiler, 1932, Maternowska, 1933), *e.g.*, when larviposition may have already commenced.

From the observations recorded above, it is clear that striking relative and perhaps absolute changes may occur in the leucocyte ratios immediately following infection, but unfortunately such neutrophilia, with left shift, and concurrent lymphocytopenia and eosinopenia is not

in itself sufficiently characteristic if it occurs in man, unless it could be linked with an immediate history of having eaten infected meat and possible gastro-intestinal symptoms.

This investigation was carried out while working under a grant from the Medical Research Council to Professor R. T. Leiper, F.R.S., to whom my sincere thanks are due.

Summary.

The differential blood count of the normal laboratory rat shows at all times a greater proportion of lymphocytes than of neutrophiles. In rats infected with 400–600 larvae there is within 2–4 days of the infection a great relative, and possibly absolute, neutrophilia with a corresponding lymphocytopenia, which becomes less marked as the infection progresses, though the percentage of neutrophiles remains above, and that of lymphocytes below, the normal average. This neutrophilia is accompanied by a moderate left shift in the Cooke polynuclear count which may persist for a considerable period. At the same time the infection is immediately followed by a relative eosinopenia or aneosinophilia which persists for 1–5 days and is probably due to the setting up of a local eosinophilia in the sub-mucosa of the intestine. Thereafter the eosinophilia becomes generalised and reaches its maximum in 7–16 days after infection, with considerable fluctuations from day to day, finally settling to a low-grade eosinophilia which may persist for some months. The degree of eosinophilia appears to be only generally related to the severity of the infection and depends on the individual host animal. Persistence of aneosinophilia is an unfavourable prognostic sign.

Monocytes and basophiles show little change during the infection, trichinosis primarily acting as a stimulus for the production of both neutrophiles and eosinophiles. In severe cases however, the stimulus may extend later to the lymphocytogenic tissue, and emphasise the production of eosinophiles only.

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Observations on *Anguillulina millefolii* (Löw, 1874) Goodey, 1932, from Galls on the Leaves of Yarrow, *Achillea Millefolium* L.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

Introduction.

Small galls on the leaves of Yarrow or Milfoil, *Achillea Millefolium* L., were first mentioned by Thomas (1873) but were investigated and described by Löw (1874) who made a new species of the causal organism under the name of *Tylenchus millefolii*. He dealt with the appearance, size and distribution of the galls, their presence on plants from spring to autumn and the ability of the parasite to revive from galls which had been kept dry from May to October. He described the general structure of the adult worms, gave particulars of their size and proportions, and, in addition to a sketch of a galled leaf, gave a drawing of the adult female and three views of the male tail.

Molliard (1904) described and figured some of the changes induced in the tissues of yarrow leaves due to gall-formation by the parasite. Marcinowski (1909) gave an account of the anatomy of the adult worms with further particulars as to dimensions and described the occurrence of galls not only on leaflets but also on the leaf axis. She also presented data for the differentiation of the adults and larvae from those of *Anguillulina tritici*. Her drawings of the adult parasite are somewhat schematic, particularly the figure of the male tail (Abb. 52, p. 119) in which no details of the shape and structure of the spicules are shown. Several authors have recorded the occurrence of galls due to this parasite on other species of *Achillea* and these are set out later on in this paper.

So far as the writer is aware, the only previous record of the occurrence of the parasite in Britain is that made by Connold (1901) who gives a photograph of galled yarrow leaves collected in the vicinity of Hastings.

The papers by Löw and Marcinowski are the only ones which deal with the morphology and anatomy of the adult worms and these are lacking in many details which are desirable for purposes of comparison with other species of the genus *Anguillulina*. Up to the present time, also, no information has been available on the life-cycle of the parasite.

The writer collected leaves of yarrow bearing galls caused by this nematode during the spring and summer of 1936 and 1937 and thus had a plentiful supply of material for observations on the structure of the parasite, its life-history and the pathology of gall-formation. The results of these investigations are presented in the following pages.

Material.

Most of the gall-bearing leaves studied by the writer were obtained from No Man's Land Common situated between St. Albans and Wheathampstead, Herts. Although yarrow is widespread on this tract of common land, the plants bearing galls are restricted to a small area, two or three square yards in extent, and have not been found on any other part although searched for carefully. An experimental infection was also successfully established on a plant of yarrow at this Institute and provided a few galls. Gall-bearing leaves were first collected at the end of June, 1936 and further collections were made during the following July, August and September. During 1937 galls were found on naturally infected plants on No Man's Land Common on May 4th and several collections were made during the ensuing months; the last lot of galls being gathered on September 21st when only one or two were obtained.

The leaves of *Achillea Millefolium* are oblong or linear in outline and are finely cut into numerous short but very narrow and deeply pinnatifid segments. The galls occur irregularly on these finely divided segments. They may be close to a tip, giving it a swollen, club-like appearance or on a side segment. Again, one or more may be found close to the base of a segment or actually in the ground tissue bordering the main axis of the leaf. Sometimes 3 or 4 galls occurring close together in such a situation cause the stem to have a twisted and knotted appearance. Up to the present, the writer has not found galls on the leaves of flowering stems, such as are mentioned by Müller (1880) and are shown in a photograph by Marcinowski (Abb. 55, p. 120), but only on leaves springing from creeping, underground stems occurring amongst fairly long grass.

The parasites occur in the gall cavity from which they may be floated out by carefully opening galls under water ; an operation best performed under a dissecting microscope. The contents of a gall vary according to the stage of development attained by the parasite and this depends on the age of the gall and the season of the year. In the large majority of cases, galls dissected throughout July, August and September are found to contain numerous adults and eggs and larvae in various stages of development. At this time, also, the adults are as a rule of two different sizes ; the larger ones, few in number, are adults of the first generation whilst the smaller and often more numerous ones are adults of the second generation. These points are dealt with in greater detail later in discussing the life-history of the parasite.

Morphology.

Dimensions :—1. Adults of the 1st generation. *Female* ; length, 1·58 mm. to 2·28 mm., α=20–28, β=8·8–12, γ=22·4–31·1, V=88%–92%. *Male* ; length, 1·33 mm. to 1·55 mm., α=27–37, β=7·8–9·1, γ=16–20, spicules, 33μ–36μ, gubernaculum, 15μ–17μ.

2. Adults of the 2nd generation. *Female* ; length, 0·93 mm. to 1·38 mm, α=28–46, β=6–10, γ=15–20, V=81·9%–86·8%. *Male* ; length, 0·85 mm. to 1·26 mm. α=30–48, β=5·7–8·9, γ=13–18, spicules, 30μ–32μ, gubernaculum, 14μ–16μ. Löw gave the length of the adult female as 1·3 mm., whilst Marcinowski gave 2·33 mm. as the maximum length of the female and 1·6 mm. for the male. It seems reasonable to suggest that these two observers were dealing with adults of the second and first generations respectively.

Adults of both sexes resemble in general appearance and structure the adults of *Anguillulina tritici* and *A. agrostis* but present a few differences from these. Adults of the first and second generation have the same structure, consequently for the sake of conciseness, a description is given of the adults of the first generation supplemented by an indication of those points of difference presented by adults of the second generation.

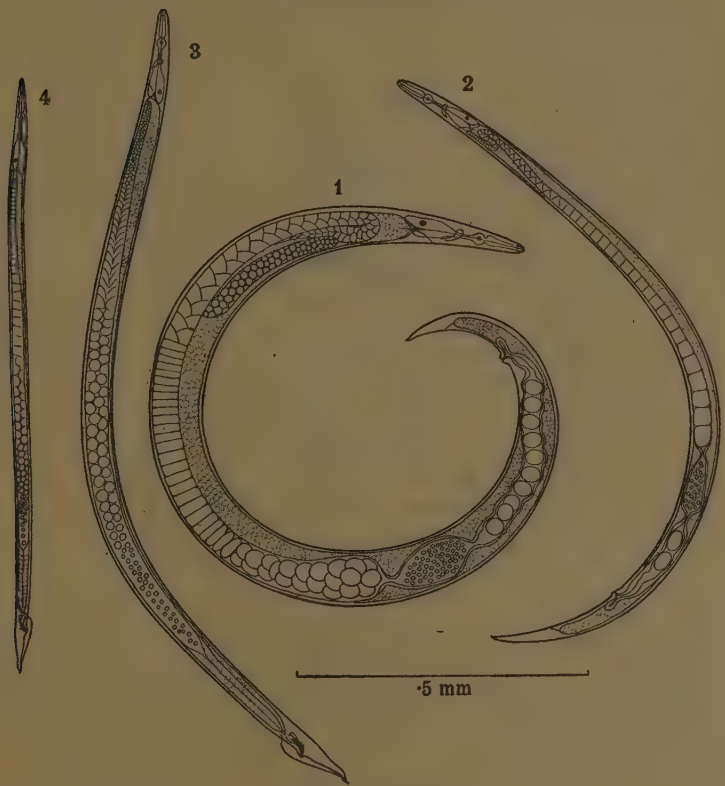
Cuticle transversely striated. Body tapering somewhat anteriorly in region of oesophagus and posteriorly in both sexes. Head a little narrower than front end of body, shaped like a rather flat cap with convex sides. Surface of head carrying the usual six radial ridges. Buccal spear 10μ to 11μ long and having the usual structure ; a conical, finely

tapering anterior half joined to a posterior cylindrical half whose base carries three knob-like swellings. Oesophagus consisting of a shortish anterior section separated by a sharp constriction from the rounded muscular bulb. Latter containing 3 well-defined crescentic thickenings of cuticular lining. Bulb succeeded by isthmus or neck region; very narrow at first, then swelling a little and finally expanding into terminal glandular region containing the three oesophageal glands. Nucleus of dorsal oesophageal gland prominent, nuclei of the two sub-ventral glands indistinct. Nerve ring crossing isthmus. Excretory pore in region of terminal part of oesophagus. Outlets of oesophageal glands into lumen of oesophagus normally situated. Intestine commencing as a conical forward process almost surrounded by terminal region of oesophagus, continuing throughout body and connected by rectum to anus.

Female. Adult females are more or less coiled watch-spring wise towards ventral side when liberated from gall and remain coiled after fixation by heat. Body broader than male, owing to great development of gonad, and tapering behind vulva to conical tail with sharply pointed tip. Vulva placed far back on ventral side of body, with rather prominent rounded lips. Vagina short, leading into uterus anteriorly and to post-vulval sac posteriorly. Latter quite small and rudimentary, consisting merely of a knob-like, backward extension of uterine wall, lumen practically non-existent. Uterus tubular with cellular walls; as a rule lying obliquely across intestine and capable of holding a good number of eggs; usually containing from 1 or 2 up to 16 or 18. Anterior end of uterus expanded into a comparatively large, somewhat flask-shaped receptaculum seminis containing numerous rounded sperms. Receptaculum seminis separated by constriction of wall from oviduct which merges anteriorly with ovary. Latter narrowing as it proceeds forwards in body, being generally reflexed twice on itself so that the anterior end often lies close up by the final part of the oesophagus.

Females of second generation differ from those of the first in the following points. Body smaller and though bent concavely towards ventral surface, the flexure is not so pronounced. Vulva situated not so far back on body being 81·9% to 86·8% as compared with 88% to 92%. The other proportions also are rather different owing to the generally smaller size of the body. Internally the uterus is correspondingly smaller and generally contains from 1 or 2 to 7 or 8 eggs at a time. Anterior end of ovary frequently found lying outstretched by terminal

part of oesophagus but some examples have been observed in which there is a short double reflexing of a small part of front part of the organ.



Anguillulina millefolii.

Fig. 1. Adult female of 1st generation. Fig. 2. Adult female of 2nd generation. Fig. 3. Adult male of 1st generation. Fig. 4. Adult male of 2nd generation. All drawn in lateral aspect and to the same magnification to show general shape and chief morphological characters.

Male. Tail tapering to a point. Caudal alae arising at about the level of heads of spicules and jutting out rather abruptly from body; not surrounding tip of tail but uniting with body just anterior to tip. Caudal papillae absent. Spicules paired and having very much the same

structure as those of *A. radicicola* and *A. dipsaci*. Seen in lateral view (fig. 6), each consists of a rather oblong front half with inwardly curving walls at anterior end. This part tapers to the curved shaft which ends in a rather blunt point. Two strengthening ridges run from the shaft into the head. Gubernaculum simple, about half as long as spicules and ending distally in a short backwardly curved process. Gonad single and of usual structure. Testis as a rule outstretched, anterior end reaching to terminal region of oesophagus. Testis increasing gradually in width and blending with vesicula seminalis. Terminal fourth of gonad with rather stout, vacuolate walls, forming vas deferens which narrows just in advance of heads of spicules to the ductus ejaculatorius. Males of the second generation differ from those of the first only in the smaller size of the body with correspondingly smaller spicules and gubernaculum.

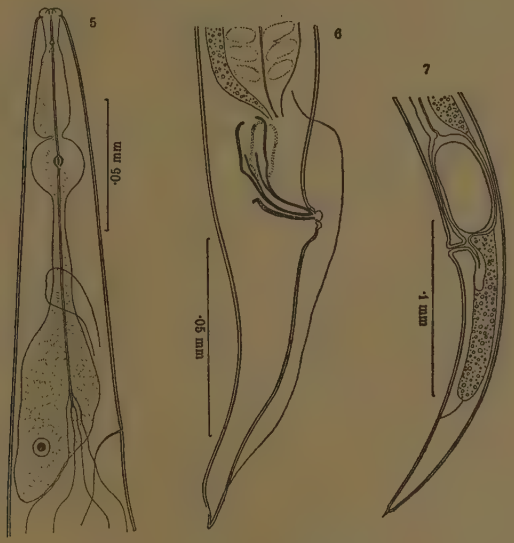
Eggs.—The eggs are rather small and more nearly round than cylindrical in outline. They measure from 42μ to 61μ long by 28μ to 35μ wide and become embryonated after being passed into the gall cavity.

Larvae.—The first stage larvae hatching from the eggs measure 0·23 mm. to 0·25 mm. long. They are comparatively stout in appearance ($\alpha=20$) and the body tapers to a rather blunt tail. The oesophagus occupies about one third of the body length. These larvae grow and a moult takes place when they have reached 0·52 mm. to 0·54mm. in length. After this growth proceeds until they attain a length of 0·65 mm. to 0·76 mm. Larvae at this second stage of development constitute the infective stage since one small gall was found to contain a single larva 0·8 mm. long. The details of the other ecdyses between the second stage larva and the adult condition have not been obtained except in the case of the final ecdysis, which, for first generation adults, takes place when the females are 1·22 mm. to 1·34 mm. long and the males about 1 mm. long.

Life-history.

Observations.—The life-history of the parasite, as revealed by the dissection of galls collected during 1936, was far from clear since a large number of them contained adults, as well as eggs and larvae in various stages of development. In 1937, however, the collection of galls was begun much earlier both from naturally infected plants and from the plant experimentally infected at Winches Farm. By this means a clearer idea of the course of the life-cycle was obtained. The experimentally

infected yarrow plant was one, found in a hedge bottom close to the Institute, whose leaves were quite free from galls. It was planted in the open at the end of two rows of *Agrostis stolonifera* which afforded it some shade and protection. Some hundreds of infective larvae, suspended in



Anguillulina millefolii.

Fig. 5. Oesophageal region of a 2nd generation adult worm under moderately high magnification. Lateral view.

Fig. 6. Male tail in lateral view, highly magnified ; showing spicules, gubernaculum and one of the caudal alae. The final region of the ejaculatory duct is purposely omitted.

Fig. 7. Posterior region of 2nd generation female in lateral view ; showing one egg in uterus close to vagina and the rudimentary nature of the post-vulval uterine sac.

water, were pipetted on to the central leaves on September 25, 1936. These larvae had been soaked out from galls which had been air-dried since August 11, 1936. Examination of the plant during the autumn and winter failed to reveal any galls on the leaves. On May 3, 1937, however, one small gall was found on a leaflet about an inch from the leaf tip. Careful dissection of this revealed 4 adult worms, 3 females and 1 male.

No eggs or larvae were present in the gall cavity. In Table I are set out the findings resulting from the dissection of a number of galls from the beginning of May to June 16, 1937.

TABLE I.—Showing contents of individual galls.

Adults of 1st generation.

Date	Total	Male	Female	Eggs	Larvae	Remarks
3.5.'37	4	1	3	Absent	Absent	Mature
4.5.'37	7	2	5	,,	,,	In final ecdysis
,, ,, ,,	3	1	2	,,	,,	Immature
,, ,, ,,	3	1	2	,,	,,	,,
,, ,, ,,	3	2	1	,,	,,	,,
,, ,, ,,	4	3	1	,,	,,	Just mature
,, ,, ,,	2	1	1	,,	,,	,, ,,
,, ,, ,,	5	3	2	,,	,,	,, ,,
,, ,, ,,	8	4	4	,,	,,	Eggs in uterus
,, ,, ,,	6	3	3	,,	,,	Mature
,, ,, ,,	2	1	1	,,	,,	Eggs in uterus
,, ,, ,,	3	1	2	,,	,,	One in ecdysis
,, ,, ,,	2	1	1	,,	,,	Immature
10.5.'37	2	1	1	Present	,,	Few eggs
24.5.'37	3	2	1	,,	,,	,, ,,
,, ,, ,,	3	2	1	,,	,,	,, ,,
,, ,, ,,	6	4	2	,,	,,	,, ,,
26.5.'37	3	1	2	,,	,,	Numerous eggs
,, ,, ,,	6	3	3	,,	,,	,, ,,
,, ,, ,,	4	2	2	,,	,,	,, ,,
,, ,, ,,	2	1	1	,,	,,	,, ,,
,, ,, ,,	6	2	4	,,	,,	,, ,,
,, ,, ,,	8	4	4	,,	,,	,, ,,
,, ,, ,,	3	1	2	,,	,,	,, ,,
,, ,, ,,	4	2	2	,,	,,	,, ,,
,, ,, ,,	3	2	1	,,	,,	,, ,,
,, ,, ,,	5	1	4	,,	,,	,, ,,
16.6.'37	3	1	2	,,	Present	Numerous eggs & larvae
,, ,, ,,	4	1	3	,,	,,	,, ,, ,,
,, ,, ,,	3	1	2	,,	,,	,, ,, ,,
,, ,, ,,	5	2	3	,,	,,	,, ,, ,,
,, ,, ,,	2	1	1	,,	,,	,, ,, ,,
,, ,, ,,	3	1	2	,,	,,	Some larvae pre-adult

On May 4, 1937 galls were obtained from plants on No Man's Land Common. Twelve of these were dissected and were found to contain adult or almost adult worms. The number of worms per individual gall varied from 2, *i.e.*, 1 male and 1 female to 8, *i.e.*, 4 males and 4 females, but the number of worms of each sex was not always equal as amongst galls dissected during May and June one with 7 adults had 2 males and

5 females, one with 6 adults had 4 males and 2 females, another with 6 had 2 males and 4 females whilst one with 5 had 1 male and 4 females.

Further collections of galls were made on May 10, 24 and 26, and on all three occasions most of them contained from 2 to 6 adults ; one was found to contain 8. A few eggs were present in the cavity of a gall opened on May 10, and eggs were found in increasing numbers in galls dissected on May 24 and 26 but no larvae were present in any of the galls opened on the last date.

More galls were obtained and dissected on June 16. These again contained from 2 to 5 adults per gall as well as many eggs and developing larvae. In one gall opened on this date, containing 1 male and 2 females, there were a good number of eggs and larvae and close examination showed that some of the larvae had grown to a stage more advanced than the infective stage and were, in fact, recognisable as being in a pre-adult condition. Galls collected on June 28 and 30 were found in most cases to contain adult worms of two sizes representing two generations of adults from one and the same gall. The larger individuals, usually few in number, were the first generation adults whilst the smaller, more numerous ones, were those of the second generation. As a rule, the females of the first generation were found to be large and the gonad was almost devoid of eggs. Occasionally adults of this generation were found in a shrunken, distorted condition whilst those of the second generation were easily recognised by their smaller size and the actively functional condition of their gonads. In addition to the second generation adults found within these galls there usually occurred considerable numbers of larvae which had not passed beyond the infective stage and, in addition, eggs laid by the second generation females.

The same picture was presented by most of the galls dissected during July, August and September, *i.e.*, one or two adults of the first generation, numerous, but very variable, numbers of adults of the second generation, infective larvae and eggs laid by the second generation females.

The number of adults of the second generation bears no recognisable numerical relation to the number of adults of the first generation. Adults of the second generation from individual galls were carefully collected and counted. They ranged in number from 7 to 116 ; the two sexes, in most cases, being nearly equally represented. Details are shown in Table II.

TABLE II.—Showing contents of individual galls as adults of each sex of 1st and 2nd generations, etc.

Date	1st gen. adults			2nd gen. adults			Infective larvae	Eggs, 2nd gen.
	Total	Male	Female	Total	Male	Female		
30.6.'37	2	1	1	7	1	6	Few	Numerous
,, ,, ,,	?	?	?	9	4	5	Numerous	,,
23.9.'36	2	1	1	11	5	6	,,	,,
,, ,, ,,	2	1	1	11	6	5	,,	,,
30.6.'37	2	1	1	14	9	5	,,	,,
,, ,, ,,	3	1	2	15	8	7	,,	,,
13.7.'37	2	1	1	15	7	8	,,	,,
,, ,, ,,	2	1	1	42	19	23	,,	,,
22.9.'37	2	1	1	44	17	27	,,	,,
28.6.'37	7	3	4	46	25	21	,,	,,
16.7.'37	2	1	1	50	27	23	,,	,,
30.6.'37	2	1	1	70	38	32	,,	,,
13.7.'37	2	1	1	80	39	41	,,	,,
23.9.'36	2	1	1	116	61	55	,,	,,

Discussion.—Not all the galls dissected from the end of June onwards contained adults of the second generation as a few were found as early as July 12, 1936 and again in July and August 1937 which contained first generation adults and infective larvae only. In most of these cases the gall was a small one and one formed the impression that the available space of the gall cavity was insufficient to allow of the development of a second generation of adults. Whether the size and capacity of the gall cavity bear any relation to the development of a second generation of adults must, however, remain purely conjectural. The galls do not vary very greatly in size and the growth of a second generation of adults may depend on quite other factors than gall capacity.

With the arrival of larvae at the infective stage, the life-cycle of the parasite may be said to have attained completion since such larvae are ready (as in the case of the second stage larvae of *Anguillulina tritici* in wheat galls) to carry on the race. Some reddish brown galls were, in fact found, early in July and August 1936, to contain rather dry, coiled, infective larvae. Others at this time were empty or contained only one or two larvae ; the bulk of them having presumably escaped.

The production of a second generation of adults within a gall thus seems to represent so much wasted or misdirected energy on the part of the parasite, since, so far as the writer has observed, eggs laid by the second generation females do not develop to the infective larval stage.

However, even when good numbers of second generation adults are present in a gall, large numbers of infective larvae are also present and these would ensure the continuance of the race.

It is difficult to determine whether there is more than one crop of galls during the course of a year. With the production of infective larvae by the early part of July, the natural term of the parasite may be considered to have been reached within the gall ; these larvae when liberated being ready to continue the life-cycle. The question arises, however, do such larvae set up fresh galls during the same season ? In other words, are the galls found throughout August and September caused by them ? The writer's observations lead him to conclude that they are not. It would be reasonable to expect to find a second crop of galls since large numbers of infective larvae must be set free from galls from July onwards. As a matter of fact the galls occurring during August and September were not at all plentiful and were found to contain two generations of adults, infective larvae and eggs laid by the second generation females. If such galls had belonged to a second crop one would have expected to find a repetition of the life-cycle with developing first generation adults, their eggs and larvae, etc. However, the possibility that some galls found late in the season may belong to a second crop cannot be entirely ruled out since one small gall opened on September 23, 1936 was found to contain a single larva 0·8 mm. long. There is, of course, no proof that this was a gall of a second crop ; it may have been a late specimen of the one and only crop.

Taking all the evidence into consideration, one seems justified in concluding that there is only one crop of galls which appear on leaves from May to September. In all probability the infective larvae set free from galls even as early as the beginning of July do not give rise to new galls later in the same year but in the year following. The life-cycle of the parasite is adapted to a plant of perennial habit. *Achillea Millefolium* continues to produce leaves throughout spring, summer and autumn and remains green through the winter and galls are produced on it throughout the warmer months of the year by larvae liberated from the previous year's galls. In this respect the life-cycle may be compared to that of *Anguillulina tritici* on wheat where the larvae set free from one season's galls give rise to a single generation of adult worms in galls formed in the ears the following year ; these adults begetting the infective larvae found in the purplish galls at the time the wheat ripens. The chief

differences between the two life-cycles are as follows. (1) In the case of *A. millefolii* a succession of galls is formed throughout the spring, summer and early autumn whereas in the case of *A. tritici* the single crop of galls are all formed at the same time. (2) In galls formed by *A. millefolii* there often occur two generations of adult worms within one and the same gall whilst in galls formed by *A. tritici* one generation only of adults is found.

Quiescence and Reviviscence.—Löw recorded the revival of the parasite from galls which had been kept dry from May to October and were then moistened. He did not specify, however, which stage of the parasite exhibited this power to withstand desiccation. The writer set aside some galled yarrow leaves on August 11, 1936, in a small glass dish so that they should become dry. Galls were removed on three occasions from these leaves and were moistened to test the power of reviviscence and to determine whether adults as well as larvae could be revived. The tests were carried out after 6 weeks, 9 months and 15 months and on each occasion infective larvae only revived and showed active motility; none of the other stages of the parasite, adults, pre-adults or first stage larvae revived. After 15 months only a few of the infective larvae became motile and it is thus possible that this parasite does not possess so great a power of resistance to prolonged desiccation as the infective larvae of *A. tritici* and *A. dipsaci*.

Symptoms.

Galls on leaflets or in the tissues bordering the main leaf axis are the only recognisable symptoms produced by the parasite. Apart from the occasional twisted or knotted appearance of a stem due to the occurrence of a gall close to the main axis, the general health of the plant and of individual leaflets seem to be in no way adversely affected by the presence of galls.

Pathology.

Structure of normal leaflet.—In transverse section an unaffected leaflet is found to be made up of the following tissues. An epidermis of lens-like cells beneath which is a loose arrangement of rather small, irregularly shaped, cells between which occur comparatively large air spaces. These cells contain chloroplasts and their nuclei are small. About midway

between the upper and lower epidermis lie the small vascular bundles, usually two or three in number, each being surrounded by a ring of empty cells composing the starch sheath. The xylem and phloem elements in

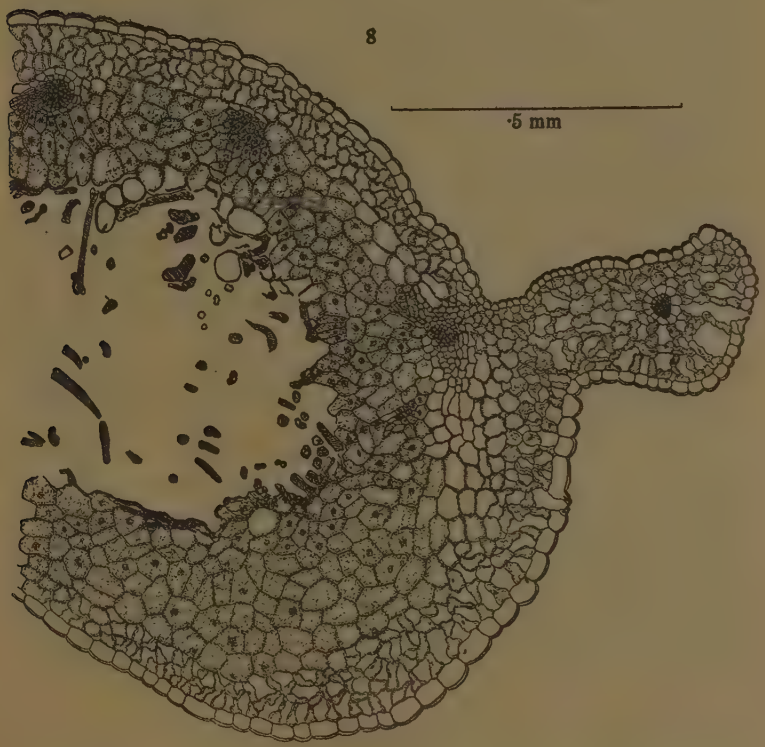


Fig. 8. Part of transverse section of gall on a leaflet of Yarrow, *Achillea Millefolium* L., caused by *Anguillulina millefolii*. On the right of the drawing is an unaffected portion of the leaflet. For description see text.

these bundles are very small. Between the bundles and occupying the central area of the leaflet are some irregularly polygonal ground-tissue or parenchyma cells but little larger than those making up the loose tissue of the rest of the leaflet. The portion on the right of Fig. 8 illustrates the foregoing remarks.

Structure of gall.—Part of a transverse section through a gall is shown in Fig. 8, from which it is at once apparent that the parasite has a profound effect on the tissues of the leaflet. The epidermal cells are rather larger than normal and must undergo considerable multiplication to accommodate the bulk of the gall itself. This is also true of the next layer of the gall which is made up of the loose, air-spaced cells as in the normal leaflet. These cells also contain chloroplasts and each has a small nucleus. These two outer layers of the gall are easily stripped off when a gall is dissected leaving a rather firm tough-walled structure below composing the main part of the gall. The drawing shows that this consists of a mass of irregularly polygonal cells surrounding a central cavity. The walls of these cells are comparatively stout but they show no secondary thickenings as in the galls caused by *Anguillulina cecidoplastes* (Goodey, 1934). No air spaces occur between these cells and it would appear that they are derived from the central parenchyma cells of the normal leaflet by multiplication and hypertrophy. Most of the cells, particularly those closer to the gall cavity, contain fairly granular protoplasm and one is probably right in considering that they constitute a broad zone of nutrient cells supplying food to the parasites occupying the gall cavity. The cells also contain each a large rounded nucleus containing two or three nucleoli. Cells immediately abutting on the gall cavity exhibit various stages of collapse and degeneration and it would appear that many of them break down during the life of the gall.

The vascular bundles become more numerous than in the normal leaflet. They are somewhat irregularly scattered in the wall of the gall and lose their symmetrical appearance. The conducting elements in them are also larger than those of the normal bundles.

Hosts.

The following species of *Achillea* have been listed as hosts of the parasite :—*A. Millefolium* L., by many authors, *A. tanacetifolia* All. (Müller, 1880), *A. nobilis* L. (Bayer, 1910), *A. Clavennae* L. (Ross, 1922) and *A. moschata* Jacq. (Trotter, 1923).

Geographical Distribution.

Thomas (1873) collected galls from *A. Millefolium* in the following regions, Thuringia, Saxony, Bohemia and the Upper Engadine. Löw (1874) found galled plants of the same species in the vicinity of Vienna.

Müller (1880) collected galls from the same host in many districts to the north, south, east and west of Berlin, in eastern Germany beyond the river Oder, in the neighbourhood of Stettin, also in Denmark not far from Copenhagen and in Sweden close to Malmö. He also received gall-bearing leaves of *A. tanacetifolia* from the Botanical Gardens at Schöneberg, Berlin. Reuter (1904) recorded the presence of galls on *A. Millefolium* in Finland. Molliard (1904) investigated the structure of galls on *A. Millefolium* presumably collected in France though he does not specify the district where they were found. Marcinowski (1909) studied the parasite from galls on *A. Millefolium* of German origin. The galls on *A. nobilis* were recorded by Bayer from Bohemia, on *A. Clavennae* by Ross from Bavaria and on *A. moschata* by Trotter from Switzerland. In addition to the foregoing, there are two records from England; first by Connold on *A. Millefolium* from the vicinity of Hastings and the record contained in the present paper from the same host in Hertfordshire. The parasite thus has a wide distribution in Europe, having been reported from the following countries:—Austria, Czechoslovakia, Denmark, England, Finland, France, Germany, Switzerland and Sweden.

Acknowledgments.

The writer desires to express his thanks to Dr. A. Smith of the Ministry of Agriculture Plant Pathological Laboratory, Harpenden, who first located the galled yarrow plants on No Man's Land Common and drew the writer's attention to them, and for his help on numerous occasions in collecting the galls.

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Leaves of Yarrow, *Achillea Millefolium* L., showing galls caused by the Nematode, *Anguillulina millefolii* (Löw). The upper end of the outside leaf on the right is irregularly thickened owing to galls in the tissues of the stem axis. Very slightly larger than natural size.

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Some Observations on the Nematode *Hexatylus viviparus* Goodey, 1926.

By T. Goodey, D.Sc.
(*Principal Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

Introduction.

Hexatylus viviparus, a spear-bearing nematode superficially resembling *Anguillulina dipsaci*, was originally described by the writer (1926) from a diseased potato tuber. In a second note published the same year (1926a), further details of its structure were given ; the organisms in this case being obtained from a gladiolus corm affected with an internal soft rot.

Since the appearance of these papers the writer has encountered this species on several occasions both in potato tubers and gladiolus corms. The receipt of a gladiolus corm in February, 1937, the brown rotten centre of which contained numerous specimens of the worm, provided further material for detailed observations on the structure of the worms. Some of the decayed central tissues of the corm also served as an inoculum for the cultivation of the nematode, along with a fungus, on slope cultures of a malt extract agar. Subcultures from the original slopes have been made from time to time and on these the worms have been successfully maintained for more than twelve months.

The writer has found it necessary to modify his interpretation of the appearances presented by the basal swellings of the mouth spear and the observations leading to this are set forth in the present paper. At the same time the opportunity is taken for presenting certain facts on the structure of the oesophagus and on the bionomics of the worm and for discussing its systematic relationship to *Neotylenchus abulbosus* Steiner, 1931.

Morphology.

Mouth Spear.—The buccal spear is small and has a total length of 10 to 11 μ. It it composed of the two parts always found making up the spear in members of the genera *Anguillulina*, *Aphelenchoides*, *Aphelenchus* and *Heterodera* namely, an anterior conical half which fits like a cap on

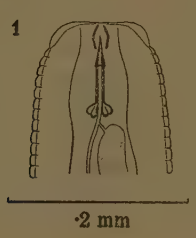
the cylindrical or tubular posterior half. The base of the latter carries the knob-like swellings or thickenings so characteristic of the spears of numerous species of the above-mentioned genera. These swellings are generally three in number ; being, in fact, the thickened bases of the three minute rods which compose the cylindrical second part of the spear.

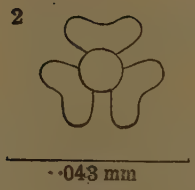
In *Hexatylus viviparus*, however, the writer claimed that there occurred six basal swellings instead of the usual three and in fig. 2 of his original paper figured four of the six. Further detailed observations necessitate the modification of this view as to the number of the swellings.

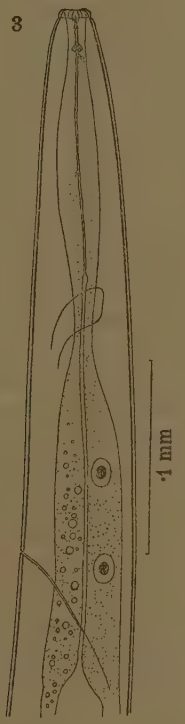
Living adult worms were mounted in the usual way in a drop of water on a glass slide ; the coverslip over the drop being sealed down with candle wax. By gentle pressure on the coverslip with the point of a needle, some of the worms were ruptured and in the resulting outflow of the body contents, the spear was carried outside the body. In two or three cases the base of the spear was uppermost and it was possible to examine it under an oil-immersion objective. It was then seen that there are in reality only three basal swellings, but that on the outside of each there is a median hollow or concavity which imparts to each swelling a bi-lobed appearance.

On other occasions living worms were closely examined under very high magnification. In mounting these specimens the film of water was thin and the coverslip pressed sufficiently tightly on the worms to prevent any active bodily movement. The heads of the worms were, however, free enough to be able to move a little, either up towards the coverslip or down towards the slide. By prolonged watching of such specimens it was possible to focus rapidly down into the depth of the head as it was directed vertically towards the coverslip and thus to see the three bi-lobed basal swellings of the spear. The median hollow running down the outside of each basal knob is sufficient to give rise to an appearance of six swellings, but the observations recorded above show that it is an appearance only since the hollow is not deep enough to give rise to six separate knobs. The rounded bases of the lobes are, however, quite distinct and separate and in worms as normally mounted, so as to lie either on the right or the left side of the body, four of the six lobes are readily discernible under moderately high magnification ; a fact which led the writer to state originally that the spear carries six basal swellings. The above points are illustrated in figs. 1 and 2.

Head Sectors.—The examination of heads of living worms when directed towards the coverslip, as described above, also made it possible to count the number of radial sectors composing the head. These were found to be eight in number, not six as stated in the writer's original paper.







Hexatylus viviparus.

Fig. 1. Head in lateral aspect, highly magnified, showing two of the three bi-lobed swellings at the base of the spear.

Fig. 2. Free-hand drawing of spear base to show the three bi-lobed swellings. This is the appearance presented by the basal swellings when seen from the front by focusing into the depth of the head. The circle in the centre represents the cylindrical shaft of the spear.

Fig. 3. Oesophageal region in lateral aspect.

Oesophagus.—The general appearance and structure of the oesophagus have been dealt with in the two earlier papers. Its most noteworthy feature is the absence of a median muscular bulb such as is commonly found in the oesophagus of most species of *Anguillulina*. A few details of its structure, which have become apparent during the observations on quiescent forms under high magnification, are worthy of mention. Fig. 3 shows the oesophageal region in lateral aspect. The fore part of the oesophagus is spindle shaped, becomes narrow in the neck region (which is crossed by the nerve ring) and then expands into a rather elongate and ill-defined terminal part. In many examples, particularly in adult worms, this part is very difficult to differentiate from the beginning of the intestine. There does not appear to be any distinct rounding off of the posterior end of the oesophagus so as to make a clavate swelling such as is found in most species of *Anguillulina*. On its ventral side one frequently finds numbers of fat-globules which further tend to obscure its connection with the beginning of the intestine. On its dorsal side the tissues have a more glandular appearance and here it has been possible to find two large nuclei; probably the nuclei of oesophageal glands. The nucleus of the third gland cell has not been located with certainty, though it is probably present.

With regard to the lumen of the oesophagus, the following points may be noted. From the base of the spear and throughout the spindle-shaped fore part the lumen is very narrow. At about the end of the fore part it becomes distinctly wider and maintains its width through the neck and the whole of the terminal region. Its final expansion into the lumen of the intestine seems to mark the junction of oesophagus and intestine. The duct from the dorsal oesophageal gland opens into the lumen in the usual place just behind the base of the spear. About halfway down the fore part of the oesophagus one can see in living specimens, under high magnification, a break in the ventral wall of the lumen which represents the opening of the subventral oesophageal glands. At all events, it corresponds in position to the opening of these glands in species of *Anguillulina* and its occurrence was reported in the writer's second note on the species (1926a).

Bionomics.

One or two facts may be put on record in regard to the question whether or not *H. viviparus* is a true parasite. The writer has so far regarded

this nematode as a semi-parasite or a saprophage rather than an obligate parasite and there is no reason to change this view since the occurrence of the worms in a gladiolus corm, in the present instance, tends to support rather than to discredit it. The corm sent to the writer from the Ministry of Agriculture Plant Pathology Laboratory, Harpenden, was one of five, all of which were suffering from the same kind of internal decay. Four of the corms contained no nematodes of any kind ; they were present only in the fifth example sent to the writer. *Hexatylus viviparus* could not, therefore, be responsible for the disease symptoms found in all the corms. On the other hand, there is very good evidence that the internal rot of the corms was caused by a parasitic fungus, a species of *Botrytis*.

Some of the spongy brown material from the corm was inoculated on to three slopes of 2% malt-extract agar which were incubated at room temperature. After a week the cultures showed a good growth of mycelium both in the substance and on the surface of the medium. The *Hexatylus viviparus* introduced along with the inoculum also grew well in and on the agar so that by the end of a week numbers of adults were found actively moving in the cultures and large numbers of eggs had been laid. Twelve days after inoculation, hundreds of larvae had hatched from the eggs, and it was clear that the medium was proving a congenial one for growth and reproduction of the nematodes. Fungus and nematodes have been subcultured together at various times by the inoculation of further malt agar slopes and living worms are present on one of these at the time of writing, *i.e.*, more than twelve months after the original culture was set up.

These facts confirm the writer in the opinion that *H. viviparus* is not a true obligate parasite since it has proved amenable to cultivation on an agar medium along with a fungus. It seems more reasonable to regard it as a species which, in all probability, feeds on fungal hyphae. In support of this view it may be mentioned that the writer has frequently seen worms with their heads closely applied to fungal hyphae, but has not actually observed the spear being inserted into them, as described by Linford (1937), an observation scarcely possible under the conditions of culturing in test-tubes. It would thus come into line with *Aphelenchoides parietinus*, *A. fragariae*, *Aphelenchus avenae* and *Anguillulina intermedia*, all of which have been shown to be feeders on fungal hyphae by the recent work of Christie and Arndt (1936), Christie and Crossman (1936), and Linford (1937). Such a view accounts for its ability to grow

well in diseased corms and on agar cultures in both of which there is an abundance of food available in the form of fungal hyphae.

Systematics.

Neotylenchus abulbosus was first described by Steiner (1931) from diseased potato tubers obtained from ship's stores coming from England, Norway and New Brunswick; also from carrots from England and Sweden and from strawberry plants, suffering from " yellows " or " xanthosis " from California and Germany. Comparison of Steiner's description and drawings with those given by the writer in his earlier papers on *Hexatylus viviparus* (*l. c.*) reveals the remarkably close similarity of these nematodes. They agree in shape, size, shape of head, character of the buccal vestibule, shape of oesophagus, shape of tail and position of the vulva. In both the single gonad is anterior and there is no post-vulval uterine sac. The only differences between the two forms noted by their respective describers are the number and character of the basal swellings of the mouth spear and the number of radial sectors composing the head. It may be noted, in passing, that Steiner and Buhrer (1932), in a short paper in which they describe the male of *Neotylenchus abulbosus*, admit the close resemblance of *Hexatylus* and *Neotylenchus*.

It seemed to the writer that the points of difference, if such existed, could be settled by a comparison between the worms recognised by American workers as *N. albulbosus* and those called *H. viviparus* by the writer. An opportunity for such a comparative study was rendered possible by the receipt from Dr. Gerald Thorne of several specimens of *N. albulbosus* which had been obtained from diseased sugar-beet at Chino, California, as described in a brief note by Thorne and Price (1935). About 20 examples of the nematode were received from Dr. Thorne. They were preserved in formalin and had been taken from the solution in which some of the affected sugar-beets had been stored. A preliminary examination of the worms in the liquid in which they were received showed that they were in a good state of preservation and that most of their structural features, including the mouth spear, were visible. After being washed in distilled water for a day they were transferred to weak glycerine and were finally mounted in glycerine jelly. Examination of the worms showed that in shape, size, appearance of head, shape of buccal vestibule, oesophagus and tail, position of excretory pore and vulva, they agree

in all respects with Steiner's description of *N. abulbosus* and also with the writer's account of *H. viviparus*. Moreover, they were sufficiently well preserved to show that the mouth spear was similar in size and structure to that of *H. viviparus* and that it was possible to discern, under high magnification, the bi-lobed character of its three basal swellings; four of the six lobes being readily countable.

In describing the mouth spear of *N. abulbosus* Steiner said: "its basal swellings or knots are very characteristic, each having a small outward-pointing, curved process for the attachment of protruder muscles." The spear with two of these processes is shown in his fig. 1b. What Steiner interprets as an outwardly pointed process attached to each basal knob is, in the writer's view, an appearance due to each knob being bi-lobed.

It has already been pointed out that the head of *H. viviparus* is made up of eight radial sectors, in which respect it agrees with *N. abulbosus*, the head of which was shown by Steiner to consist of eight sectors. In view of all the foregoing considerations, the writer is of the opinion that *N. abulbosus* and *H. viviparus* are morphologically indistinguishable and accordingly *N. abulbosus* should be considered as synonymous with *H. viviparus*. *Neotylenchus* was transferred by the writer (1933) to the genus *Hexatylus* with *H. abulbosus* as a species additional to *H. viviparus* but in view of the conclusion stated above the two species should be considered as identical.

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Journal of Helminthology, Vol. XVI, *No.* 2, *May*, 1938, *pp.* 117-120.

On a Dermatitis in Malays caused by the Cercariae of *Schistosoma spindale* Montgomery, 1906.

By J. J. C. Buckley, D.Sc.
(*Milner Research Fellow, London School of Hygiene and Tropical Medicine.*)

A skin infection called by the local Malays "sawah itch" has been known in Negri Sembilan, Federated Malay States, for some years, where it has been recorded from widely distributed areas in this State and also from the rice-fields adjoining the Tampin-Malacca road. It is essentially associated with padi-cultivation and is especially prevalent during those periods when the soil is being ploughed or "chankolled" prior to planting with rice seedlings, which occupation necessitates workers spending hours each day with their legs submerged in the mud and water of the sawah. (Sawah = swamp or tract of ground covered with water.) I am indebted to Dr. J. W. Field of the Institute for Medical Research, F.M.S., for permission to quote the following extract from his unpublished report on "sawah itch." From enquiry into several individual cases he elicited the information that the condition followed a similar course of events in each case, viz., "A worker enters the sawah in the morning, works there for some hours and in the evening has a severe itching in the ankles and legs, rarely on the back of the hands and never anywhere else. If he does not re-enter the sawah on succeeding days the irritation dies down and no external lesion is produced; but if he goes in day after day the constant irritation results in scratching and a scratch dermatitis is produced which becomes secondarily infected and shows lesions indistinguishable from those seen as a result of scratching and sepsis following severe skin irritation of any form."

Previous investigations as to the cause of the itch were based on the theory of a cercarial infection of some kind, which were justified by the fact that the Malays themselves put the blame on water snails, which sometimes are very common in the sawah; and they point out that only in localities where there are snails is there "sawah itch." In the Annual Report of the Institute for Medical Research, F.M.S., of 1929 (p. 45) reference is made to the laboratory attempts to produce dermatitis

experimentally with cercariae obtained from snails from the infected area, which, however, proved negative. The present investigation of the problem was undertaken at the suggestion of Dr. A. Neave Kingsbury, Director of the Institute, and in November 1935 infected localities were visited by the writer accompanied by the Health Inspector, Abdul Majid bin Lassim, whose help and knowledge of the local conditions was invaluable. Snails were collected at Repah and Kepis which were later identified as *Planorbis exustus* and *Limnaea* sp.

Cercariae were obtained only from the *Planorbis* and proved to be of three different kinds, (1) an Echinostome cercaria, (2) a cercaria with an oral stylet and (3) a furcocercous cercaria of the typical Schistosome form. Neither (1) nor (2) caused any irritation when placed on the forearm in drops of water. The Schistosome cercariae, however, which were present in 8 out of 89 *Planorbis* from Kepis, produced an itching sensation within five minutes after application. The itching, although acute, was not intolerable and the skin was not scratched or rubbed in any way. It began before the drops of water had evaporated to a thin film, so that penetration of the skin was apparently taking place without the assistance of the surface tension of a film of water such as is required by hookworm larvae. After about half-an-hour inflamed spots 1–2 mm. in diameter appeared on the skin. The itching continued for 2 to 3 hours and then passed away. Two days later the inflammation disappeared, but the lesions were still visible as minute elevations on the skin of about 2 mm. in diameter. No further changes occurred and fourteen days later the skin was normal again.

Experimental Infection of Mice.

Cercariae of different species of Schistosomes resemble one another so closely that their identification on morphological characters alone is somewhat hazardous. Except for minor differences the cercariae used in the above skin infection seemed identical with that described by Soparkar (1921) as the cercaria of *Schistosoma spindale*, but confirmation of this was sought by infecting mice with the cercariae and allowing them to develop to the adult stage in which they would be identifiable with more certainty. Accordingly ten laboratory-bred mice were exposed to infection on the 20th November, 1935, by placing them in glass vessels with about 5 mm. depth of water containing large numbers of the cercariae. At different dates up to three months later five of these mice were dissected and in

every case adult worms were found in the liver or mesenteric veins, and were identified as *S. spindale*. The life-cycle of this Schistosome in *Planorbis exustus* was worked out by Liston & Soparkar in 1918, so that further remarks on this aspect of the parasite are unnecessary here.

The normal hosts of *S. spindale* are ruminants, including goat, sheep and oxen, and the Indian buffalo has been experimentally infected by Fairley & Jasudasin (1927). The normal host of this parasite in the localities where " sawah itch " occurs, and probably the host which is mainly responsible for the infection of *Planorbis exustus* in those localities, appears to be the buffalo. Not only is it the most likely one from circumstantial evidence, for the buffalo is employed in the cultivation of the padi fields, but the writer has found adult *S. spindale* post-mortem in the mesenteric veins of a buffalo in Negri Sembilan.

From Dr. Field's account of the development of the dermatitis in Malays and from the writer's experimental dermatitis, it is apparent that the lesions caused by the cercariae are of little importance in themselves. It is the sepsis caused by scratching which constitutes the principal ill-effect. Control of the condition, therefore, appears to depend upon personal hygiene rather than on attempts to eradicate the sources of the primary causal organism, which would be difficult, expensive and probably impermanent. The Malays themselves have in some cases found a solution of the problem by wearing puttees and boots whilst working in the sawah. Probably another effective method of prevention would be to inculcate the habit of washing the legs and hands after leaving the sawah and then applying an antiseptic.

Human schistosomiasis has never been recorded from Negri Sembilan and it can be fairly safely assumed that the cercarial infection causing " sawah itch " is purely a dermal one. It is of interest to note, however, that there are several records in the literature of cases of human schistosomiasis, which, from the characteristic spindle-shaped eggs found in the faeces or urine, have been diagnosed as being due to *S. spindale* or some very closely related species. These cases were recorded by Christophers & Stephens (1905) in India and by Cawston (1925, 1927 and 1930) and Porter (1926) in Africa. As a potential human parasite, therefore, *S. spindale* must be regarded with suspicion, especially in localities where man, the accidental host, is in close association with normal hosts such as the buffalo, and is frequently exposed to infection with this parasite.

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On *Culicoides* as a Vector of *Onchocerca gibsoni* (Cleland & Johnston, 1910).

By J. J. C. Buckley, D.Sc.

(*Milner Research Fellow, London School of Hygiene and Tropical Medicine.*)

The problem of the mode of transmission of *Onchocerca gibsoni*, the cause of " worm nodules " in cattle, has been the subject of investigation during the past thirty years. In Australia, where the diseased condition in beef, produced by this parasite, has a wide distribution and is a source of great economic loss, much valuable experimental work has been carried out to determine the method of spread of the disease and the possibilities of its control or eradication. The solution of the problem has so far, however, persistently evaded the grasp of investigators ; but in its pursuit much information has been acquired, and the negative results which have attended the studies on the life-cycle of the causal parasite have reduced the possible lines of research to a considerable extent. In recent years the elucidation of life-cycles of closely related species has done much towards clarifying the situation with regard to *O. gibsoni* The value of the use of analogy in such investigations has long been recognised and the earlier workers on the transmission of *O. gibsoni* were influenced by it, but were handicapped in this respect by the lack of available knowledge of parallel life-cycles. Some of the earlier work on this problem included explorations into the possibility of direct transmission, either by contact of one animal with another, or through the soil, but this line was soon abandoned by most workers for experimental and theoretical reasons in favour of the theory of an intermediate host. The life-cycle of *Dracunculus medinensis*, a parasite somewhat distantly related to *Onchocerca*, offered a parallel which, apart from this flimsy taxonomic link, could only be justified in its

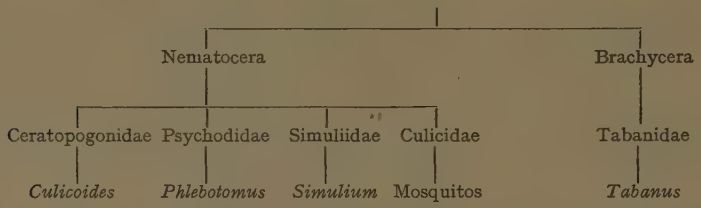
application by the similarity in the location in the host of the adult parasite, namely, in the subcutaneous tissues. A strong objection, however, to the theory of *Cyclops* as an intermediary of *O. gibsoni* as well as of *D. medinensis* was the apparent absence of any method whereby the larvae could gain the exterior of the host and infect these water-crustaceans; and although Breinl (1913) demonstrated that the larvae under certain conditions can penetrate the skin of cattle in small numbers and get to the exterior, the *Cyclops* theory was also abandoned. Leiper (1911) furthermore had pointed out that the zoological affinities of *O. gibsoni* indicated that it was a species which required an insect intermediary, not an aquatic arthropod such as *Cyclops*. This theory was early recognised as offering the most promising field of research and upon it has been based the principal experimental work on this subject. At the outset, however, a strange difficulty was encountered. The larvae of related filarial worms, such as *F. bancrofti* in man and *Dirofilaria* in the dog, were known to frequent the blood stream of the host, but repeated efforts to demonstrate the larvae of *O. gibsoni* in the blood of infected cattle failed to give conclusive results. Nevertheless, the theory of the biting insect intermediary still held good and experiments with various species of insects were carried out. (It was not until 1920 that Montpellier & Lacroix, by discovering the larvae of *O. volvulus* in the skin of African natives, provided the clue to the habitat of *O. gibsoni* larvae.) In the large number of available cattle-biting insects another difficulty arose, for there was little evidence to indicate even to what group of insects the vector would be likely to belong. Gilruth & Sweet (1911) produced a strong argument that lice were the probable vectors, but their researches (1912) with these insects gave negative results. Experimental work with other species of insects has been based mainly on circumstantial evidence and up to the year 1920 much negative information was forthcoming with regard to many species of the common cattle-infecting genera such as *Tabanus*, *Stomoxys* and *Lyperosia*; mosquitos and ticks have also received considerable attention as possible vectors. Summaries of the results of these investigations have been published from time to time, by Johnston (1911), Nicoll (1914), Johnston & Bancroft (1921) and Mohammed (1931), so that a detailed historical review of the work is unnecessary here. Reference must be made in some detail, however, to certain investigations which are relevant to the present one. One of the most important contributions in the second decade was

by Cleland (1916) and others who worked at Milson Island, New South Wales. The disease being endemic in cattle in this locality there seemed to be every prospect of narrowing down the number of possible vectors by experimental work with the limited number of insects on the island. A careful survey of the cattle-biting insect fauna revealed mosquitos, Tabanids, *Stomoxys*, Leptids, lice and one species of *Culicoides*. The conclusions drawn from the work at Milson Island were that either mosquitos or Tabanids were the most likely vectors, Tabanids more so than mosquitos. The other insects were excluded on experimental or circumstantial evidence. In subsequent investigations Tabanids received more attention than any other species, but Johnston & Bancroft (1920) failed to find these insects naturally infected although thousands were dissected, nor did feeding them on *Onchocerca* nodules produce any positive results. Their work with Muscids was also negative. Cilento (1923) reported the experimental production of *Onchocerca* nodules by implantation with nematode larvae from the heads of Tabanids. This result has not, however, been confirmed and was not claimed to be conclusive. In the meantime a hitherto unconsidered vector was coming into the picture. Robles (1919) had suggested that *Simulium* was the transmitter of human onchocerciasis in Central America. In 1926 Blacklock proved that the vector of *Onchocerca volvulus* was *Simulium damnosum* and suggested (1927) that some species of *Simulium* might prove to be the vector of *O. gibsoni* in Australia. Cleland (1927) however, repudiated this suggestion, pointing out that *O. gibsoni* was transmitted in Australia in localities which were free from any species of *Simulium*. The importance of the relationship between the genus *Simulium* and the life-cycle of *Onchocerca* was further established when Strong (1931) proved three species of *Eusimulium* to be carriers of *O. caecutiens* in Central America and Steward (1937) incriminated *Simulium ornatum* in the transmission of *O. gutturosa* in cattle in England; but in view of Cleland's strong argument that *Simulium* is not implicated in the transmission of *O. gibsoni*, Steward's (1935) discovery that the genus *Culicoides* acts as intermediary for *O. cervicalis* in the horse is of paramount significance and throws new light on the *O. gibsoni* problem.

Discussion.

If *Simulium* be excluded as a possible vector, yet taking into consideration its biological connection with the genus *Onchocerca*, it is

reasonable on biological grounds to look for the vector of *O. gibsoni* in a group of insects related to the Simuliidae.



The search would, therefore, be confined to the families of biting insects included in the Nematocera and thus the Tabanidae are excluded. With regard to the Culicidae, experimental work with species belonging to this family, although not exhaustive, has failed to justify any belief that mosquitos might act as vectors. Furthermore, as Heydon (1927) wisely remarked as to the probable vector : " This fly must be one which imbibes not only blood but tissue fluid. For this reason it seems unlikely that a mosquito is concerned, since its slender and delicately wielded piercing organs cause the minimum of laceration and abrasion, and possibly are inserted directly into a small vessel." The elimination of the Culicidae therefore seems justifiable, after which there remain the Psychodidae and Ceratopogonidae for consideration. For reasons which will become apparent later in this paper, *Phlebotomus* may be disregarded as a possible vector, so that only the Ceratopogonidae remain. More than one genus of biting flies is included in this group but attention is immediately drawn to *Culicoides* which is known to act as intermediary for filarial parasites, namely, *F. perstans*, *F. ozzardi* and *O. cervicalis*. In connection with Heydon's theory as to the nature of the vector, it is interesting to note that the mouth parts of *Simulium* resemble those of *Culicoides* very closely.

Possibly owing to its small size—it is difficult to believe that scarcity is a contributory reason—*Culicoides* has received little attention from investigators in the search for a vector of *O. gibsoni*. The few references to it in the literature of *O. gibsoni* investigations however, furnish no evidence as to why it should have been excluded. McEachran & Hill (1916) and Dickinson & Hill (1916) mention *Culicoides subnitidus* (?) in connection with their valuable experiments with uninfected calves and an infected herd in the Northern Territory. Their results served

to exclude a large number of insects from the list of possible vectors, for, although these were observed frequently biting two uninfected calves in an open roofed pen situated not far from the infected herd, the calves did not become infected with *O. gibsoni*. They noticed, however, that certain species of *Tabanus* rarely bite stock under a roof, so that these still remained suspect. The information regarding the *Culicoides* sp. is somewhat meagre, but such as it is, it suggests that like the *Tabanus* species in question it also remained suspect, for McEachran & Hill found it on the herd of infected cattle in the open paddock but do not mention having found it on the calves which remained uninfected. Possibly *C. subnitidus* (?) is also a species which does not bite under a roof or perhaps only flies very short distances, and, if either of these hypotheses be correct, it still remains a possible vector.

Dickinson & Hill's references to " sand flies " (presumably *Culicoides*) present in the open roofed pen in similar experiments are rather vague. Cleland, Dodd & Ferguson (1916) observed *Culicoides molestus* around cattle on Milson Island and included this species in their extended observations on the cattle-biting insects on the island, but for reasons which are not apparent they concluded: " It does not seem at all likely that this insect can be the transmitter." They dissected a few of these insects for natural infections but found none.

The case for *Culicoides* as the vector of *O. gibsoni* therefore appears to be a very strong one, in view of the available evidence. It is supported (1) by the analogy of the development of *O. cervicalis* in *Culicoides nubeculosus*, (2) on biological grounds by the exclusion of *Simulium* and the other Nematocera, (3) by the fact that previous investigations in which *Culicoides* was included tend to support the theory that it is a vector rather than otherwise.

Investigations with *Culicoides* and *O. gibsoni* in Kuala Lumpur.

The researches of Gilruth & Sweet (1912) and Sweet (1914) have shown that Malaya is an important centre of bovine onchocerciasis and that *O. gibsoni* was introduced into northern Australia from this source. The presence of *O. gibsoni* in the Malay Peninsula was recorded by Daniels (1904) and Sweet (1914) in cattle at Kuala Lumpur, where the present investigation was carried out. Enquiry at the abattoir in Kuala Lumpur in 1935 and personal observations there revealed that the infection was

still common, and that 90-95% of locally bred cattle harboured it. Cattle are imported into Kuala Lumpur for slaughter from the native State of Kelantan, from Siam and from Bali, Dutch East Indies, and these also are infected to a high degree. Data are lacking concerning cattle from Malay States other than Kelantan, as few were imported during the writer's stay. The very high percentage of infection in local animals, *i.e.*, cattle born in Kuala Lumpur and its environs and from Selangor State generally, was of particular interest. The degree of infection can be roughly gauged from the numbers of nodules removed from animals slaughtered at the abattoir. The routine procedure at the abattoir is to cut away the nodules from carcases if the nodules are few in number and easily dealt with and then the meat is passed. Only in case of heavy infestation is the carcase condemned. A record of the number of nodules thus obtained is kept in the abattoir returns and is here tabulated for the period January 1936 to July 1937.

Source of cattle	Number slaughtered	Number of nodules removed	Average number of nodules per head
Local	3,012	5,268	1·75
Kelantan	707	1,715	2·42
Siam	616	2,122	3·44
Bali	596	4,191	7·0

These figures are of principal interest as a record of the comparative degree of infestation between cattle from different localities. The numbers of nodules were actually greater than appears in the table, for only large-sized ones were recorded. The greatest number of nodules seen by the writer in one animal was 49, in a locally born bullock about 20 years old. The nodules are typical in appearance and occur in the usual sites, namely, the brisket and inter-costal spaces. Rarely, other sites are infected, such as the shoulders. Buffalos have apparently never been found infected in the abattoir, but nodules occasionally occur in buffalos imported from Kedah for use in the lymph station at the Institute for Medical Research, Kuala Lumpur. Adults removed from these nodules were identified by the writer as *Onchocerca cebei* Galliard, 1937.

From these preliminary observations at the abattoir it was apparent that *O. gibsoni* must be an endemic infection in local cattle and the high

percentage of infection indicated that it was being freely transmitted. In the search for a vector it was decided to collect insects from infected living animals and dissect them for natural infections with filarial larvae and also to keep alive insects which had fed on infected cattle and dissect these for developing stages of larvae.

Diagnosis of O. gibsoni *in live cattle.*—In order to be sure that cattle from which insects were collected were infected with *O. gibsoni*, the skin was examined for the presence of the microfilariae in preference to palpation for nodules, as the latter method was found to be unreliable. Except in emaciated cattle, nodules in the deeper tissues could easily remain undetected. For examination of the skin the procedure was as follows:—A small area on the flank of the animal was first shaved. The skin was then pinched up between thumb and forefinger and a very thin slice of skin, ·1 to ·3 mm. thick was removed with a sharp razor blade. It was found advisable to rope the hind legs together while doing this operation although in the majority of cases the animals were almost insensitive to it. The slice of skin, which was usually about 10 to 20 sq. mm. in area was examined on a slide or in a watch glass with a few drops of normal saline. Microfilariae, if present, would emerge from the skin within a minute or two and swim about actively in the fluid. A convenient method of mounting them for examination was devised by mixing the saline containing them with an equal quantity of fresh blood and smearing the mixture over the slide in the form of a thick blood film. This was then dried, dehaemoglobinised, fixed and stained in the usual way. If the number of larvae in a piece of skin was to be counted, the skin was left soaking in the saline for about 12 hours, during which period it was found the majority of the larvae would emerge. This method of examination was carried out on various local herds of cattle and the positive results obtained in nearly every case corresponded with the high percentage of nodule infection observed at the abattoir. The microfilariae from the skin were identified as those of *O. gibsoni*, but the possibility of the presence of other species of *Onchocerca* could not be entirely excluded. *O. armillata* was recorded in cattle at Kuala Lumpur abattoir by Tuck (1904) who found this parasite, or manifestations of it, in the aortae of 62% of local and imported animals. A recent examination of 26 *local* cattle showed 5, or 19·2%, of these to be infected with *O. armillata*. It is not known whether the microfilariae

of this species inhabit the blood or skin tissues of the host. From the dimensional difference between the microfilariae of *O. armillata* and *O. gibsoni*, in conjunction with the results of the experimental work with insects, it was concluded that although *O. armillata* larvae may have been present in small numbers in the skin or blood of the cattle, this was not a serious complicating factor in the experimental work. From the literature and from personal observation it appears that the microfilariae of *O. armillata* are considerably larger than those of *O. gibsoni*. Tuck (1904) states that *O. armillata* larvae are 0·29 to 0·32 mm. long. The writer found uterine larvae from Kuala Lumpur material to be 0·35 to 0·37 mm. long and from material from Jerusalem the range was 0·34 to 0·38 mm. In the case of *O. gibsoni* the writer found microfilariae from Kuala Lumpur nodules to be 0·23 to 0·27 mm. long. *Onchocerca* microfilariae from the skin or blood of the cattle used in the experimental work did not exceed 0·28 mm., nor did any of the microfilariae picked up with a blood meal by the insects concerned in the experimental work. The examination of the blood of the cattle in question revealed a microfilaria which was apparently that of *Stephanofilaria kaeli* Buckley, 1937, a common parasite in the epidermis of cattle in Kuala Lumpur causing sores in the legs. The microfilariae were very scanty in numbers and easily distinguishable morphologically from *O. gibsoni* larvae. *O. gibsoni* larvae were also found in small numbers in blood films from infected cattle, but it is probable that they were in reality derived from the skin tissues, from which they were expressed or emerged at the site of the puncture. *Elaeophora poeli*, although very common in buffalos in Kuala Lumpur, is apparently extremely rare in cattle. Its larvae furthermore are easily distinguishable from those of *O. gibsoni* and would have been detected in the insect infections. It can be safely said, therefore, that the predominant microfilarial infection in cattle in Kuala Lumpur is derived from the adults of *O. gibsoni*.

Intensity of skin infection with O. gibsoni *larvae*.—The choice of certain animals for use in the experimental work with *Culicoides* depended upon the degree of infestation with larvae in the skin, for it was thought that insects which fed upon cattle whose skin was heavily charged with larvae would naturally be more likely to pick up some of these during a blood meal. Accordingly an attempt was made to measure the degree of skin infestation by counting the number of larvae which emerged from pieces

of skin cut from living animals and measured as to their surface area. As far as was possible, the skin slices were cut at a uniform thickness and were taken from the same location in different animals. The degree of infestation, expressed as the number of larvae from 1 sq. mm. of skin was found to vary very much in a group of 13 cattle. When the test was repeated on the same group, little correlation was found between the two results, so it was concluded that there must be one or more variable factors affecting the results. These factors appeared to be the slight variations in the thickness of the skin slices together with the fact that even in a very small area of skin the larvae were unevenly distributed. This was demonstrated as follows: Eight small slices of skin were taken from a spot just behind and a little below the left shoulder of a cow from an area of about 3 sq. inches. The skin slices were measured and the total number of larvae emerging from each was counted with the following result.

Area of skin slice, in sq. mm.	15·0	19·5	17·0	26·0	9·5	18·0	20·0	11·0
Number of larvae recovered	510	210	140	105	50	31	19	14
Average number of larvae per sq. mm.	34	10·8	8·2	4	5·2	1·7	1·0	1·3

The inevitable slight variation in the thickness of the skin slices (·1 mm. to ·3 mm.) could not wholly account for such a difference in larval distribution as 34 per sq. mm. and 1 per sq. mm., assuming that the larvae were evenly and homogeneously distributed in that part of the animal from which the skin was removed.

Since the distribution of larvae in small areas of skin has a bearing on the question of the insect vector, it was investigated further by a method which practically eliminated the thickness factor. A slice of skin about 10×5 mm. in area was cut from the dorsum of a heavily infested animal and this was trimmed down with a razor blade to a rectangular piece measuring $8 \times 1{\cdot}5$ mm., which was of uniform thickness throughout. This was cut into 8 pieces roughly $1 \times 1{\cdot}5$ mm. in size, each of which was isolated in a drop of saline and its total content of larvae was counted. The accompanying diagram represents the strip of skin and the figures

are the number of larvae recovered from each piece.

8 mm.

1·5 mm.							
12	16	33	59	167	65	31	0

Here the uneven distribution is well illustrated. Of some interest is the concentration of larvae in one place with a gradual decrease in numbers further away from it. A second piece of skin, taken from the same part of the same animal, was cut into a rectangle 4×2 mm. and then in the same way divided into smaller pieces 1 mm. square as in the second diagram. Here again the figures in each square represent the number of larvae recovered.

4 mm.

2 mm.			
22	29	149	135
48	75	38	66

It can be seen from this diagram that a large number of larvae may be present under 1 sq. mm. of skin. The figure 149 per sq. mm. is considerably greater than that obtained by Heydon (1927) by the method of serial sectioning of skin from slaughtered animals, which gave 15–40 larvae per sq. mm. The distribution of larvae in pieces of skin smaller than 1 sq. mm. could only be satisfactorily observed by sectioning. Transverse and tangential sections were accordingly made of skin slices identical in every way with those in the diagrams above. In the transverse sections it was seen that there was a concentration of larvae immediately under the epidermal layer (Plate I, Fig. 1) at a depth of ·05 to ·2 mm. and deeper than ·2 mm. the larvae were scanty. In the tangential sections the larvae were seen irregularly distributed in the spaces between hair follicles, with a definite tendency to clumping together in a mass in certain spots whilst in other spots not far distant there would be few or no larvae at all (Plate I, Fig. 2).

Cattle used in the investigation.—In spite of these aberrations in the distribution of the larvae, repeated examinations of the skin of cattle revealed that certain animals were more heavily infected with larvae than others, and these were employed in the routine collecting of insects and in the experimental work. At first a 20 years old cart bullock with

two palpable nodules on the flank and a good skin infestation with larvae was purchased and installed at the grazing ground attached to the Institute for Medical Research in October 1935. Unfortunately this animal died the following month and subsequently insects were collected from selected animals from a herd three miles outside Kuala Lumpur on the Klang Road. During the latter part of the investigation it was found more convenient to work with animals in the herd comprising the Kuala Lumpur Dairy Farm which was fairly close to the laboratory.

Cattle-biting insects in Kuala Lumpur.—Preliminary collections of insects from cattle during the last three months of 1935 produced species of *Stomoxys*, *Lyperosia*, *Tabanus*, *Chrysops*, *Haematopota*, *Culicoides*, *Lasiohelea*, *Haematopinus*, ticks and mosquitos. Neither *Simulium* nor *Phlebotomus* were found, nor did they appear at any time during the following 19 months in which frequent collecting was done at various times of the day and night. Furthermore, with regard to *Simulium*, I am indebted to Mr. H. M. Pendlebury for the information that *Simulium* only occurs in the Malay Peninsula at elevations above 3,000 ft. in localities such as Fraser's Hill, Cameron's Highlands and Taiping Hill, far removed both in distance and elevation from Kuala Lumpur. This evidence bears out Cleland's contention that *O. gibsoni* can be endemic in localities where *Simulium* is unknown. In view of this, and for the reasons already outlined it was decided to concentrate on *Culicoides* and other cattle-biting insects closely related to it which might appear.

Method of collecting Culicoides.—Collecting was done directly from the cattle from about 7.30 to 9.30 a.m., or in the evening an hour or two before sunset. It was during these periods that *Culicoides* were most abundant on the cattle, being extremely scarce during the heat of the day. As the cattle were grazing at these hours, practically all the collecting was done in the field, unless for some special purpose it was done inside the pen. Collecting at night was carried out during the latter months of the investigation and required a special technique which will be described later. For day-collecting test-tubes were used, the method being simply to place the mouth of the tube over an insect as it alighted on the cow or was making its way between the hairs to get into the skin surface to feed. When tubed the insect was shaken down to the bottom and a small plug of cotton wool was inserted nearly to the bottom, leaving a space of about half an inch. A second insect being caught, the process was

repeated and so on until finally the tube held about 8 flies each in a separate compartment. After practice, and by skilful manipulation, keeping a finger over the mouth of the tube to prevent the flies escaping, as many as ten flies could be enclosed in each compartment, thus economising both in test-tubes and in time, for each test-tube could thus be made to hold over 50 flies. More than this number was found inadvisable as they would not live for more than a few hours in such a confined space. The great majority of the flies were collected from the flanks or under parts of the body of the cows and the test-tube method was the most efficient for this. Flies which were engorged were particularly easy to catch for, being heavy with blood they fell to the bottom of the tube and were incapable of escaping. For collecting from the dorsal parts of a cow the test-tube method was not efficient and a suction collector was used. This was devised specially for the purpose by Mr. E. P. Hodgkin, B.Sc., of the Institute for Medical Research. It consisted of a tubular glass container 6 ins. long by $1\frac{1}{2}$ ins. in diameter, corked at each end, one of which was provided with a rubber suction bulb and the other with a piece of curved glass $\frac{1}{4}$ in. tubing. The connecting tube for the bulb was covered with fine mesh gauze to prevent the flies being sucked into the bulb, and over the internal end of the curved tube a piece of stiff paper was fixed, forming a slit-like aperture which offered the minimum chance of the flies escaping or being expelled from the container when the bulb was squeezed. A buffer of cotton wool at the bulb end of the container prevented the flies being killed or damaged by the impact due to the force of suction. A considerable blast of air was required to suck the flies into the container and for this reason the narrow $\frac{1}{4}$ in. tube had to be used instead of a wider one or a funnel mouth which would have made manipulation easier.

The numbers in which *Culicoides* came to feed on cattle during the periods mentioned were surprisingly high. By the test-tube method, as many as 500 could be collected in one hour on a favourable day. An average catch was about 250 flies, less than 100 was poor, and over 500 exceptionally good. The majority of these would be unfed females, but a skilled collector could exclude these and catch only those which were gorged with blood. These could be seen emerging between the hairs from the skin surface after a blood meal, and after a pause of a second or two would fly away. Males were never taken on cattle. No definite

correlation was observed between weather conditions and the prevalence of the flies, but rain during night as a general rule meant good collecting the following morning. Windy or rainy conditions greatly reduced the numbers of flies in the collections. It is of interest to note here that, unlike *Tabanus* or *Stomoxys*, the bites of *Culicoides* seemed to cause no inconvenience to the cattle.

Night collecting of Culicoides.—For night collecting an electric torch was required which could be fastened on to the head or leg of the collector in order to leave the hands free for catching. The light was directed on to the flank or brisket of the cow and flies were tubed as they alighted on the illuminated part. The best time for collecting at night was found to be between 11 p.m. and 2 a.m. Here again, as in the day collecting, there was great variation in the numbers caught on different nights. Sometimes an hour's collecting would yield only half-a-dozen flies, whereas on one occasion 576 were taken between 12.45 and 2.15 a.m. A curious fact about this night collecting was that the great majority of the flies caught had already had a blood meal and were never actually observed feeding. As these flies comprised species which differed in many ways from the day collections, it may be assumed that normally they feed in darkness and are merely attracted to the light on the cow and do not come there to feed. For this reason it was not known whether they had fed upon the cow on which they were caught; or that they had fed on cow's blood, although this was very probable. Flies were noticed to come in "waves" of abundance, *i.e.*, after scanty collecting for about 15 minutes, they would suddenly come in such numbers that they could not be collected quickly enough.

Anaesthetising and sorting Culicoides.—Collections made in the morning were dealt with in the laboratory within a few hours after they were caught. Evening and night collections could safely be left in the test-tubes until the following day with only a very small mortality. The flies were released from the tubes into a large glass chimney closed at each end with fine-mesh muslin. The phototropism of the flies facilitated this operation greatly. In order to separate fed from unfed flies or isolate different species into different chimneys it was necessary to anaesthetise them for a short period without actually killing them. They were then transferred rapidly from the chimney to a large solid watch glass half full of water. The sorting was done under a binocular

microscope, each specimen being removed by lifting it off the surface of the water on which it was floating, on the tip of a mounted needle, and then transferred to another watch glass which was covered. The flies revived usually within 2 or 3 minutes after being chloroformed so that the sorting had to be done rapidly. If it was not completed within this time, the watch glass was covered when signs of life appeared and chloroform vapour was introduced with a pipette. The flies were remarkably resistant to repeated small doses of chloroform and recovered full activity without any apparent ill-effects.

Method of keeping Culicoides *alive.*—The sorted flies were put into separate chimneys if they were engorged with blood and it was intended to keep them alive for examination for developing stages of a possible *Onchocerca* infection. For this purpose the chimneys were kept in darkness in an incubator at laboratory temperature. The necessary conditions of humidity were provided by a tray of water at the bottom of the incubator. For nourishment the flies only required raisins which were cut in half and stuck on to the muslin of the chimneys. It was not found necessary to provide them with a blood meal, for under these conditions they remained alive for periods up to 3 weeks which proved adequate for the experiments. The only precaution necessary was to prevent raisin juice flowing down the muslin to the inside of the chimney and forming a pool there in which some of the flies invariably would be caught and perish. Fungal growths on the raisins were inevitable and necessitated fairly frequent replacement of the raisins or the muslin.

Dissection of Culicoides.—The most satisfactory instruments for dissecting *Culicoides* were found to be rustless steel headless entomological pins, such as are used in mounting *Culicoides* and small flies, fixed in adaptable aluminium handles. At first the flies were dissected singly in a drop of saline on a slide. Later on, when it was realised what large numbers needed to be dissected, the process was speeded up a lot by dissecting 3 flies on one slide at a time, each fly in its own drop of saline. Developing stages of filarial larvae when found were fixed in glycerine alcohol and mounted in glycerine. Gut infections, *i.e.*, microfilariae picked up with a blood meal were fixed on the slide after removal of the insect débris, by addition of a little fresh blood, dried and treated as an ordinary film. The blood in the gut of *Culicoides* was usually in a semi-solid mass when the fly was dissected and it had to be mixed thoroughly

with the saline in order to free any microfilariae which might be present.

Preliminary results of dissections.—During the first few months of the investigation it was not properly realised that there was a large number of different species of *Culicoides* in the collections. The number was thought to be about four and these were given a numeral nomenclature until their correct names would be forthcoming. They were worked with in the manner described above, being sorted into fed and unfed lots, the fed flies being kept alive for varying periods before being dissected and the unfed ones were dissected immediately for possible natural infections

Species of *Culicoides* and *Lasiohelea* from cattle in Kuala Lumpur.

C. anophelis Edw.
C. anophelis v. *flavescens* Macfie, 1937.
C. boophagus Macfie, 1937.
C. buckleyi Macfie, 1937.
C. daleki Smith & Swaminath.
C. geminis Macfie, 1937.
C. gentilis Macfie.
C. insignipennis Macfie, 1937.
C. malayae Macfie, 1937.
†*C. orientalis* Macfie.
C. oxystoma Kieff., 1910.
C. peregrinus Kieff.
†*C. pungens* de Meij.
†*C. raripalpis* Smith.
C. shortti Smith & Swaminath.
C. similis C. I. & M.
C. sumatrae Macfie.
Lasiohelea stimulans (de Meij.).
**L. lefanui* (Carter).
**L.* sp. near *L. nigeriae* I. & M.

(†Concerning these species Dr. Macfie says—"More than a single species may be included under these names, but it would be inadvisable, I think, to attempt to separate them until the range of variability of the species is better known, and, in some cases, males are available for examination."

* For correct identification of these species a comparison of males would be necessary).

with filarial larvae. The results were very disappointing. A filarial larva was found in the head or thorax of a few of the flies, but in such a small percentage of them, even in those that had been kept alive after feeding on infected cattle, that it was thought it must be a natural infection with some species other than *Onchocerca*. On the other hand, there was the possible explanation that there were in reality more species of *Culicoides* than four, and that a more accurate knowledge of them might show that the filarial infection was in reality present in a higher percentage in a restricted number of species. A more detailed examination of the flies revealed that there were over a dozen species in the experimental material. Actually, during the period of the investigation the total number of species collected from cattle in Kuala Lumpur alone was about twenty. These were identified by Dr. J. W. S. Macfie who has published descriptions (1937) of certain species which are new and redescribed others which were not well known.

Relative incidence of different species of Culicoides *and* Lasiohelea.—The following figures are the summarised numbers of flies, sorted into their different species, which were caught in February, March and April, 1936, and are the results of morning collections on 30 different days and evening collections on 16 days. As well as indicating the relative incidence of species, the figures also serve to illustrate the large number of these flies which may be found on cattle, for the total of 9,343 represents the result of 46 collections (usually made by a single collector) which gives an average catch of over 200 flies. The time taken in making the collections varied from 1 to 2 hours.

	Morning	Evening	Totals
C. oxystoma ...	4,942	937	5,879
C. pungens ...	899	615	1,514
C. shortti	255	375	630
L. stimulans ...	188	90	278
L. lefanui	143	87	230
C. orientalis ...	98	98	196
C. paripalpis ...	133	37	170
C. peregrinus ...	131	15	146
C. anophelis ...	99	46	145
C. buckleyi ...	122	21	143
C. daleki	6	6	12

The figures for *C. peregrinus* must be taken as including a small proportion, less than 10%, of *C. sumatrae* which was not differentiated as a separate species at this time.

With regard to night collecting, the following figures, which are the totals of all species taken at night in May 1937, represent about 10 hours' collecting on seven different nights by a single collector.

C. shortti	410	*C. sumatrae*	13
C. peregrinus	225	*C. anophelis*	6
C. orientalis	194	*C. gentilis*	3
C. oxystoma	109	*C. daleki*	2
C. raripalpis	66	*C. buckleyi*	1

Although most of the day species are represented in the night collections, with the notable exception of *C. pungens* and *Lasiohelea* spp., their relative incidences are strikingly different. Thus, *C. shortti*, *C. peregrinus* and *C. orientalis*, which are relatively scarce in the day collections, are the commonest species at night. *C. oxystoma*, by far the commonest species in the day collections, takes only fourth place at night.

Biting habits of Culicoides.—With the knowledge that there were at least 12 different species to be dealt with, which after practice were quickly recognisable, the work of dissecting was continued. But the rate of infection with the filarial larva already mentioned still remained obstinately below 1%. It was noticed, however, that there was a small difference in the natural infection rate and the experimental infection rate in *C. oxystoma*, but in order to prove that this difference was significant it was realised that it would be necessary to dissect very large numbers of the flies. At this period there was a tendency to exclude *C. pungens* from the experimental work and dissecting, for the following reasons. The theory that the location of *Onchocerca* nodules on the body, in the case of *O. volvulus* and *O. caecutiens*, is dependent upon the biting habits of the insect vector, has considerable support. On the assumption that it is correct, it seemed legitimate to apply it also in the case of *O. gibsoni* and to argue that the vector of this species must be an insect which habitually bites cattle in the region of the brisket. Conversely, a species of insect which habitually bites on other parts of the body might theoretically be excluded as a possible vector. A species which bites indiscriminately might also be excluded, if with less certainty. Accordingly collections of *Culicoides* were made on four consecutive days, from 7 to 9 a.m., and were divided into two lots, (1) those collected from the brisket region and from the ventral surface generally, (2) those collected from the neck and along the vertebral column. The collections

were then analysed as to their species and the results are here tabulated. Further, a collection was made from a cow inside a roofed open pen in order to ascertain if the location of the cattle affected in any way the incidence of species.

Species	Location of Cattle	Number collected		Total collected	
		Ventral	Dorsal	Ventral	Dorsal
C. oxystoma ...	Field	768	73	856	114
	Pen	88	41		
C. pungens ...	Field	79	304	83	311
	Pen	4	7		
C. shortti ...	Field	53	6	57	8
	Pen	4	2		
C. orientalis ...	Field	2	36	2	36
	Pen	0	0		
L. stimulans ...	Field	12	3	17	3
	Pen	5	0		
L. lefanui ...	Field	0	38	0	39
	Pen	0	1		
C. buckleyi ...	Field	22	0	22	0
	Pen	0	0		
C. varipalpis ...	Field	11	0	11	0
	Pen	0	0		
C. peregrinus ...	Field	2	0	5	0
	Pen	3	0		
C. daleki	Field	0	0	1	0
	Pen	1	0		

It was concluded from these figures (1) that *C. oxystoma* prefers biting on the under parts of cattle, but not exclusively, (2) that *C. pungens* on the other hand prefers biting on the dorsal parts, again not exclusively, (3) that *C. pungens* prefers to bite in the open rather than in

the pen, (4) that the dorsal-biting habit of *C. pungens* and its preference for the field is probably connected with phototropism, (5) that *C. shortti* resembles *C. oxystoma* in its ventral-biting habit. The figures representing the other species are probably insufficient to be conclusive in every case, but it would appear that *C. orientalis* and *L. lefanui* are dorsal-biters and that the remainder are ventral-biters.

Since *C. pungens* formed a large proportion of the collections, its exclusion on the grounds of its biting habit lightened the work of dissection greatly, but that this was an unwise course became apparent later when it was found that it was more efficient at picking up larvae from the skin during a blood meal and gave a higher percentage of experimental infections than *C. oxystoma*. It was ultimately concluded that *C. pungens* is a vector of *O. gibsoni*, and in the light of this conclusion it becomes necessary to re-examine the possible cause of the ventral location of the nodules of *O. gibsoni*, and this point will be discussed subsequently.

Morphology of filarial larvae found in *Culicoides*.

The filarial infections found in certain species of *Culicoides* fall into three groups which appear to represent distinct species and are separated on the basis of the morphology of the mature larvae dissected from the head or thorax of the flies. They will be referred to, for convenience, as Types A, B and C.

Type A is a smallish larva varying in length from ·34 to ·46 mm. and with almost uniform thickness of ·012 to ·015 mm. throughout the length of the body. The anus is about 0·02 mm. from the tip of the tail which is bluntly rounded and lacking in papillae. The anterior end is also bluntly rounded and plain. It was found in the head or thorax of 3 different species of *Culicoides*, *C. oxystoma*, *C. buckleyi* and *C. pungens*, and is illustrated in Fig. 1 and Plate II, Fig. 3.

Type B is larger than Type A, with a length varying from ·54 to ·75 mm. and a maximum width of ·015 to ·02 mm. The maximum width is in the region from the middle of the body to about the posterior third from which the body tapers gradually to the oral end and to the anus. Behind the anus the tail narrows somewhat more abruptly to the rounded extremity. The tail is ·025 to ·035 mm. long. Lateral caudal papillae were faintly discernible in one or two specimens but are not characteristic enough to be of value in identifying the larva. Sub-oral papillae were also seen in one

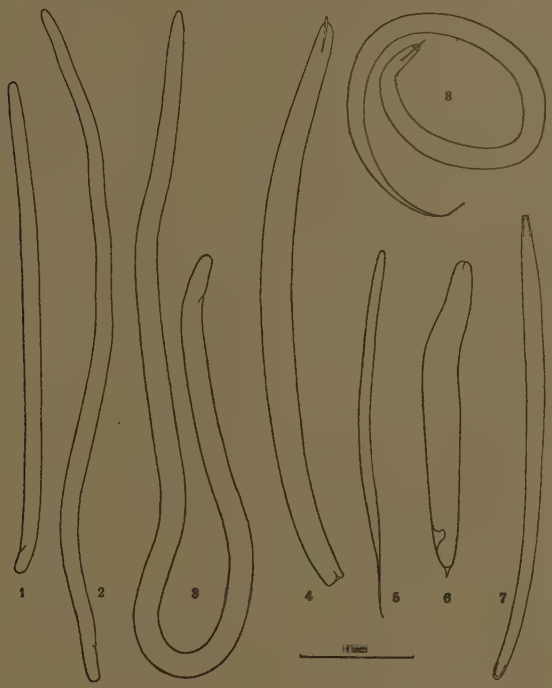
specimen, but these also are unreliable features. The differentiation of oesophagus and intestine was usually too vague, even in living larvae, to be described accurately. The characteristics of this larva are its dimensions, especially the tapering of the anterior half of the body. It was found in the head or thorax of five species of *Culicoides*, *C. pungens*, *C. oxystoma*, *C. shortti*, *C. orientalis* and *C. buckleyi*. It is illustrated in Fig. 2 and Plate II, Fig. 4. A larva, apparently of this type, but in rather poor preservation, was once found in *C. peregrinus*.

Type C larva resembles Type B in its general appearance and shape but is distinguished from it by its much greater length, ·95 to 1·15 mm. with a maximum breadth of about ·02 mm., and a tail length of ·04 mm. It was found in a very minute percentage of only one species of *Culicoides*, *C. oxystoma*, and is illustrated in Fig. 3 and Plate II, Fig. 5.

Type B larva was suspected early on as being the larval stage of *O. gibsoni* and throughout the investigation was the only larva which gave any response in the attempts to infect flies experimentally with *O. gibsoni* larvae. No conclusion could be arrived at concerning Type C as it was so extremely rare. Type A was definitely eliminated, on experimental grounds, as a possible larva of *O. gibsoni*.

Non-filarial nematode infections in Culicoides.—Various nematode infections were encountered during the dissections whose origins are obscure, but which are here classified as "non-filarial" on account of their morphological difference from any of the stages typical of larval filarial development. These were usually eliminated without difficulty, but a developing stage of a Mermithid infection closely resembling a "sausage" larva was at first mistaken for one (Fig. 6 and Plate II, Fig. 8). Later stages of this Mermithid attain a length of 5 mm. and occurred in the abdomens of *C. oxystoma*, *C. pungens*, *C. peregrinus* and *C. buckleyi*. A massive infection with hundreds of small larvae (Fig. 5 and Plate II, Fig. 7) was found several times in the abdomen of *C. orientalis*; and in this species and in *C. pungens* a larva with an oral stylet (Fig. 8 and Plate II, Fig. 6) was found coiled up in the head on a few occasions. A peculiar larva with an oral stylet and truncated posterior (Fig. 4 and Plate II, Fig. 9) was found once in the thorax of *C. oxystoma*. *C. shortti* was twice found infected with many of the larvae illustrated in Fig. 7 and Plate II, Fig. 10. It is characterised by its rounded

posterior extremity, thick cuticle and squarish oral extremity with papillae. Its maximum breadth is in the anterior half of the body.



Mature filarial larvae in *Culicoides*. Fig. 1. Type A. Fig. 2. Type B. Fig. 3. Type C. Figs. 4–8. Non-filarial nematode larvae in *Culicoides*.

Results of dissecting different species of *Culicoides* and *Lasiohelea*.

In the following account of the results of dissections each species is dealt with separately and the total number of specimens dissected and the total number of infections found during the period of the investigation are lumped together in the case of each species without reference to the time of the year or the locality in which the insects were collected and experimented with, nor to the different animals on which the flies were collected.

The dissected flies are classified under three headings :—(1) specimens which were dissected soon after being collected in order to determine the nature and percentage occurrence of any filarial larvae which might be present as *natural infections*, (2) specimens which had had a blood meal on *O. gibsoni* infected cattle just before being caught and which were kept alive for some days before being dissected, in order to determine (a) the nature of any filarial larvae which might be present, (b) whether there was any correlation between the length of time the flies had been kept alive and the stage of development of the filarial larvae present, and (c) whether the percentage in these flies differed significantly from the natural infection percentage, (3) specimens which were dissected soon after having had a blood meal on infected cattle, in order to determine what percentage of flies would pick up the microfilariae from the skin as a result of feeding on the infected cattle. These three headings will be referred to in abbreviated form in the tables as (1) Natural Infections ; (2) Experimental Infections ; (3) Microfilarial Infections.

Culicoides pungens de Meij.

This has already been referred to as one of the commonest day-biting species on cattle, with a preference for biting on the dorsal parts of the cattle. It was rarely found in cattle inside a roofed pen and was never taken at night. Extensive searching in various types of localities failed to reveal the breeding place of this species, so that the specimens dissected were all of necessity " wild flies." The predominant larval infection found was Type B. Type A was only found in two of the 5,404 specimens dissected and Type C was never found at all in this species. In view of the great scarcity of Type A and the absence of Type C, it is assumed that the immature larvae found both as natural and experimental infections are referable to Type B and are included as such with the mature infections in the following table. Microfilariae were found in the gut of 8 out of 1,523

Infection of *C. pungens* with Type B filarial larvae and with microfilariae of *O. gibsoni*

	Natural Infections	Experimental Infections	Microfilarial Infections
Number dissected	3,734	1,670	1,523
Number infected	13	16	8
% infected	0·348	0·958	0·52

flies dissected immediately after a blood meal on infected cattle, and were identified as the microfilariae of *O. gibsoni*.

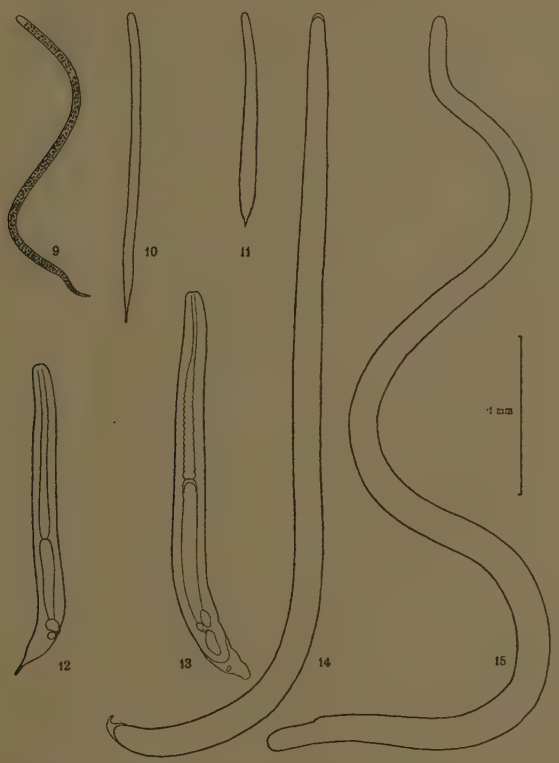


Fig. 9. Microfilaria of *O. gibsoni*. Figs. 10 & 11. Early stages in *C. pungens* 4 days after infective blood meal. Figs. 12 & 13. Post-" sausage " stages 4 days and 52 hours respectively after infective blood meal. Fig. 14. Pre-infective stage in *C. pungens* 5 days after infective blood meal. Fig. 15. Mature larva from head of *C. pungens*.

Although the natural and experimental infection rates are extremely low the higher experimental infection rate is noticeable, and a statistical treatment of the figures shows that the difference between the two infection rates is significant, χ^2 being equal to 8·0. Furthermore it is of considerable interest and probably significant that the infection rate with microfilariae in the gut agrees so closely with the natural and experimental

infection rates. Thus, theoretically speaking, the percentage of flies which pick up microfilariae with the blood meal should equal the percentage of flies found infected with developing or mature larvae some days after the blood meal. But, since " wild flies " were used in the experimental infections, the natural infection percentage is superimposed on the experimental infection percentage and therefore the latter, *i.e.*, 0·958% should exceed the " pick-up " rate, *i.e.*, 0·52% by the natural infection rate, *i.e.*, 0·348%, which in practice it does approximately.

Further evidence that the larvae in the experimental flies have developed from microfilariae picked up with the blood meal is seen in the approximate correlation between the stage of development of the larvae and the time which the flies were kept alive after the blood meal. The details of the sixteen experimental infections are here tabulated.

Experimental infections in *C. pungens*.

Date of dissection	Duration of infection Days	Stage of development of larvae	Number of larvae	Location of larvae in fly
18.xii.36	2	" sausage "	1	thorax
28.i.37	2	,,	1	,,
18.xii.36	2	,,	1	,,
14.i.37	3	† pre " sausage "	2	,,
21.xii.36	4	" sausage "	1	,,
2.i.37	4	* mature	1	,,
12.ii.37	5	mature (in sheath)	1	,,
5.ii.37	5	mature	1	,,
12.ii.37	5	,,	3	head
14.v.36	6	,,	5	thorax
13.v.36	7	,,	1	,,
28.xii.36	9	,,	1	,,
21.iv.37	11	,,	1	,,
7.v.37	11	,,	1	head (?)
20.iv.37	11	,,	1	head
21.iv.37	11	,,	1	,,

(* This was either a natural infection or a case of very rapid development, as the larva was full-grown, 0·75 mm. long.

† An active undeveloped microfilaria was also found in this fly. Apparently a case of very slow development.)

The number of larvae constituting an infection is of interest for in the majority of cases it consisted of a single larva, 3 and 5 being exceptional. Of the thirteen natural infections, 10 consisted of a single larva in each case, and 3 of two larvae in each. These figures compare well with the

numbers of microfilariae picked up with the blood meal. Of the eight cases of microfilarial infection, 7 consisted of a single microfilaria in each and one of 3 microfilariae.

Owing to the very low infection rate and the consequent scarcity of material, it was impossible to follow the course of the development of larvae in the body of the fly and information as to the morphological changes is lacking. From the above table, however, it may be concluded that the time of development from microfilaria to infective stage lies between 6 and 12 days.

It may be concluded, therefore, with regard to *C. pungens* that it is capable of picking up the microfilariae of *O. gibsoni* from the skin of infected cattle while engorging itself with blood and that these microfilariae develop to mature infective larvae, designated Type B in this investigation, which lodge in the head of the insect after a period of 6 to 12 days' development in the body of the insect.

"*Culicoides oxystoma* Kieff 1910.

This species was the commonest in the collections made on cattle in the morning and evening, but was rare at night. A voracious feeder, like *C. pungens*, there was never any scarcity of specimens " with blood," and the bulk of the work centred about this species during most of the investigation. Over 14,000 of this species were dissected altogether, which comprised about half the total number of all species of *Culicoides* and *Lasiohelea* which were dissected. The majority of the *C. oxystoma* dissected for experimental infections were of course " wild flies," but as a result of finding the natural breeding places of this species it was possible to employ a limited number of laboratory-bred specimens in the experimental work. Altogether 177 laboratory-bred specimens were fed

Infections of *Culicoides oxystoma* with filarial larvae Types A, B and C, and with unclassified immature filarial larvae.

	Natural Infections	Experimental Infections
Number dissected	9,006	5,335
Number infected with mature larvae		
Type A	4	—
Type B	8	13
Type C	2	4
Number infected with unclassified immature larvae	9	—

from chimneys on the skin of an infected cow. Unfortunately none of these became infected, but this was not unexpected in view of the minute percentage of " wild flies " which were naturally infected or which were found infected after an infective blood meal. In the dissections of " wild flies " of this species all three types of filarial larvae were encountered and their incidence is tabulated on the previous page.

Assuming that the 9 immature natural infections consisted of Types A, B and C in the ratio of 4 : 8 : 2, they may be divided up approximately into 2 Type A, 6 Type B and 1 Type C. This gives the following totals and percentages.

Infections of *Culicoides oxystoma* with filarial larvae Types A, B and C, and with microfilariae of *O. gibsoni*.

	Natural Infections	Experimental Infections	Microfilarial Infections
Number dissected...	9,006	5,335	2,259
Number infected			4=0·177%
Type A	6=0·066%	—	
Type B	14=0·155%	13=0·24%	
Type C	3=0·033%	4=0·075%	

Type A will be discussed in more detail in connection with its occurrence in *C. buckleyi* which it infected to a relatively high degree. Concerning Type C, little can be said or concluded on account of its very low infection rate. Here again, as in the case of *C. pungens*, the experimental infection rate with Type B is higher than the natural infection rate, but the difference between them is not significant, the value of χ^2 in this case being only 1·4. There is some correlation between the microfilarial infection rate and the natural and experimental infection rates, but this is not so well defined. Of the thirteen experimental infections, 11 were of one larva each and the remaining two had 2 and 4 larvae in each. These were dissected from insects which had been kept alive for 5 to 9 days and were all mature. Six were head infections and the remainder were from the thorax. The four microfilarial infections consisted of 1, 1, 1 and 4 larvae respectively.

It may be concluded therefore concerning *C. oxystoma* that a very small percentage of these flies which feed on infected cattle are capable of picking up the microfilariae of *O. gibsoni* during the blood meal and that in a

correspondingly small percentage of such flies the microfilariae develop to mature infective stages after a variable period of 5 to 10 days.

Culicoides orientalis Macfie.

This species closely resembles *C. pungens* in its wing markings and was often confused with the latter during the earlier part of the investigation. It was relatively scarce in the morning and evening collections but quite abundant at night. The results of dissections of this species recorded here were obtained from specimens the great majority of which were collected at night. These gave a surprisingly high " pick-up " infection rate. Thus, of 513 dissected soon after an infective blood meal, 13 were found to have microfilariae in the gut. Seven of these consisted of a single microfilaria in each, three had two microfilariae, one had three microfilariae and in one fly 13 microfilariae were found. In the latter instance, however, some of the larvae were dead or degenerating and it may be concluded that they were not all picked up at one blood meal but were probably acquired as the result of several infective blood meals and that some of them had failed to continue developing and were degenerating. This unusually high " pick-up " rate, compared with that of *C. pungens* and *C. oxystoma* did not justify the expectations aroused of a consequent high development percentage. Thus, of 297 caught after an infective blood meal and kept alive for an adequate period, only 3 were found on dissection to harbour mature larvae of the usual Type B. Of 562 dissected for natural infection, only two were positive. These results are here tabulated.

Infection of *Culicoides orientalis* with Type B filarial larvae and with microfilariae of *O. gibsoni*.

	Natural Infections	Experimental Infections	Microfilarial Infections
Number dissected	562	297	513
Number infected	2	3	13
% infected	0·35	1·01	2·5

The higher experimental infection rate is again evident in this species, but statistically it is not significant, the value of χ^2 in this case being only 1·4. From the identification of the mature larvae as Type B however, it may be concluded that *C. orientalis* must be regarded as an intermediate host of *O. gibsoni*.

The natural breeding place of this species having been found, it was possible to use a few laboratory-bred flies in the experimental work. Twenty of these were fed on infected cattle, but were negative on dissection at varying periods after the feeding. In view of the low infection percentage obtained with "wild flies" this result is, however, inconclusive.

Culicoides shortti Smith & Swaminath.

After *C. oxystoma* and *C. pungens* this was the commonest species in the morning and evening collections, and was the most abundant species at night. Like *C. orientalis* it has a relatively high "pick-up" rate; of 518 specimens dissected soon after an infective blood meal, eleven or 2·1% were found with active microfilariae in the gut. Here again, however, the experimental infection was strangely at variance with the "pick-up" rate.

Infection of *Culicoides shortti* with Type B filarial larvae and with microfilariae of *O. gibsoni*.

	Natural Infections	Experimental Infections	Microfilarial Infections
Number dissected	1,414	800	518
Number infected	4	6	11
% infected	0·28	0·75	2·1

As in the case of *C. oxystoma* and *C. orientalis* the percentage of experimental infections is considerably greater than that of the natural infections, but the difference between the two is not significant, χ^2 being equal to 2·5.

The eleven infections with microfilariae in the gut consisted of 9 with one larva in each, one with two larvae and one with four larvae. The experimental and natural infections with developing or mature stages consisted of a single larva in each case. The conclusions to be drawn from the work with this species are the same as for *C. orientalis*.

Culicoides peregrinus Kieff.

This was one of the largest of the cattle-biting species and was common but not abundant in the morning and evening collections. In the night collections it usually formed a high proportion of the catch. Although during a blood meal it would imbibe about 3 to 4 times as much blood as *C. pungens*, yet its "pick-up" rate was very small compared with the latter

species. Of 689 dissected soon after an infective blood meal only two were found with microfilariae in the gut. Natural infections were found in 3 out of 808 dissected. Two of these were " sausage " stages and one was a mature larva 0·67 mm. long, which on account of its poor state of preservation can only doubtfully be referred to Type B. Of 67 flies dissected some days after an infective blood meal none were found infected. The breeding places of this species were found to be similar to those of *C. oxystoma*.

Culicoides sumatrae Macfie.

This species resembles the previous one very closely and was confused with it at first. It occurs in about one-tenth the frequency of *C. peregrinus* in the morning and evening collections. Attention was first drawn to it when a specimen which was dissected after a blood meal was found to have 17 microfilariae in the gut. It was thought in consequence that this might be a really effective vector which would give a high percentage of experimental infections. The numbers in which it occurred were too few, however, in the day collections to make it practicable to work with, and night collecting of *Culicoides* was first begun systematically on account of this species, in the hope that it might prove to be a night biting species and could be obtained in larger numbers. This hope was justified to some extent but experimental work with this species was disappointing, for no developing larvae were found in it, although 3 out of 20 dissected after a blood meal were found to have microfilariae in the gut in the numbers of 17, 7 and 1 respectively. It was noted, however, that many of these microfilariae were dead or degenerate, so that it would appear that although it has a relatively high infectibility with microfilariae, this species is an unsuitable host for the development of the larvae. Eighteen specimens were kept alive for several days after the infective blood meal but neither microfilariae nor developing stages were found in them. Sixty specimens dissected for natural infections were negative.

Culicoides buckleyi Macfie.

This is a small species which occurred with very variable frequency in the morning and evening and was very scarce at night. The number of specimens " with blood " in any collection was usually very small and it is probably not normally a cattle-biting species. For experimental work therefore, it was necessary to collect the unfed specimens and feed them

artificially on cattle from glass chimneys. Of 128 specimens dissected after a blood meal none had picked up microfilariae, and 52 were negative of developing stages some days after the blood meal. During the period April 1936 to January 1937, 1,416 of this species were dissected for natural infections and showed a consistent infection of over 1% with Type A larva, the actual figures being 19 infected flies or 1·34%. That these infections were not acquired as the result of feeding on cattle was proved by the repeated failure to find microfilariae in the gut or developing stages in the body of flies kept alive several days after a blood meal. Although the numbers of flies used in this experimental feeding were necessarily small, viz., 128 for microfilarial infections and 52 for developing stages, yet they were adequate in view of the high natural infection percentage in this species. It is of interest to note that 15 of the 19 natural infections consisted of mature larvae in the head or thorax, indicating a very rapid development in the fly. The number of larvae in each infection varied from 1 to 4.

Only once was Type B larva found in this species, as a natural infection of 1 mature larva in the head, measuring 0·62 mm. long.

Culicoides anophelis Edw. and *C. anophelis* var. *flavescens* Macfie.

This species and its variety were not distinguished from one another during the investigation and are considered as one in the following observations. It was taken on cattle both day and night and was not uncommon. A voracious feeder, it was at times a nuisance owing to its habit of preying on other species of *Culicoides*. If one of them were included in a test-tube along with other species it would attack and kill them, apparently by sucking the blood from the abdomens of fed flies. Owing to their light colour both of wing and body it was comparatively easy to distinguish them with the naked eye from other species during the collecting. Of 309 dissected after an infective blood meal 3 were found infected with microfilariae in the gut, and of 361 dissected for natural infections 3 contained immature larvae. Twenty-four, kept alive for some days after a blood meal were negative for developing stages.

Culicoides raripalpis Smith.

This species resembles *C. anophelis* both in its appearance and in its incidence on cattle, and was taken both in the day and night collecting, but rather more commonly at night. Unlike *C. anophelis* it was never

observed attacking other species. Of 245 dissected soon after an infective blood meal two were harbouring microfilariae in the gut, indistinguishable from the larvae of *O. gibsoni*. No natural infections, however, were found in 335 flies dissected, and six kept alive after a blood meal proved negative.

Culicoides daleki Smith & Swaminath.

Under this heading must also be included *C. similis* C. I. and M., and *C. geminis*. They were comparatively scarce and only 67 were dissected "with blood" during the investigation. These were negative, as were also 83 dissected for natural infections. Three only were kept alive after a blood meal and these proved negative.

Culicoides gentilis Macfie.

A rare species on cattle which was not distinguished from *C. malayae*, another rare species, during the investigation. Only a few of either species were worked with and only negative results were obtained. Two were dissected "with blood," five for developing stages and four for natural infections.

Lasiohelea stimulans (de Meij).

A fairly common species in the day collections but absent at night, which showed a marked preference for the under parts of the cattle, particularly along the mid-ventral line. Of 502 dissected for natural infections, all were negative. Of 145 dissected soon after an infective blood meal, one had two active microfilariae in the gut, apparently *O. gibsoni*. Of 20 kept alive for some days after an infective blood meal, one was found to have a single small "sausage" larva after four days. The small number of experimental flies was occasioned by the difficulty of keeping these flies alive even for a few days. There was usually a high mortality in the chimneys after the first day. Little can be concluded therefore as to its capability of acting as a vector of *O. gibsoni*. *Lasiohelea* sp. inq., near *L. nigeriae*, was not distinguished from *L. stimulans* in these dissections.

Lasiohelea lefanui (Carter).

A species with a rather variable incidence and less common than *L. stimulans*. It was only taken in the day collections and was apparently without any special preference for biting on particular parts of the cattle.

Of 160 specimens dissected for natural infections, one was found to have a mature larva in the thorax which was unfortunately badly damaged in dissection and could not be identified. 94 specimens were dissected soon after a blood meal but were negative. Nine were kept alive for varying periods after the blood meal but were negative on dissection.

Breeding places of *Culicoides*.

In May 1936 an attempt was made to discover the breeding places of *Culicoides*, primarily with the object of obtaining flies for experimental work, which would be known with certainty to be free from natural infections with filarial larvae, and secondarily for the reason that any information about the breeding habits of these flies might subsequently be of value from the point of view of control.

The following method of locating possible breeding places was suggested by Mr. E. P. Hodgkin, B.Sc., whose advice and help in this matter was greatly appreciated. Samples of material such as mud, slime, manure, scrapings from soil, etc., were taken from the vicinity of the cattle pen or grazing grounds, and were placed separately in large glass vessels 6 to 12 ins. in diameter by about 6 ins. in depth, and these were then covered with fine mesh muslin. Eggs or larvae of *Culicoides* if present in the material would develop to adults in one to four days and were collected from the muslin surface by means of a suction apparatus. By this method the breeding places of *C. oxystoma*, *C. peregrinus* and *C. orientalis* were found. *C. oxystoma* and *C. peregrinus* were bred out in large numbers from mud and slime taken from the sides of drains or small streamlets. *C. orientalis* and to a lesser extent *C. peregrinus* were bred out from material obtained from *old* manure heaps, *i.e.*, two or three weeks at least, whose surface was well dried by the sun but which were still moist an inch or two under the surface, where the larvae were developing. Extensive searching in other types of material failed to reveal the breeding places of such common species as *C. pungens* and *C. shortti*. *C. daleki* and *Lasiohelea stimulans* (?) were found in very small numbers in the same material as *C. oxystoma*.

Method of feeding laboratory-bred Culicoides *on Cattle.*—Having collected a number of insects from the breeding vessels, they were sorted and kept alive in glass chimneys in the usual way. It was found advisable not to attempt to feed the flies on cattle immediately after they had bred out, but to give them raisins for one day, water only on the second day and to

attempt the experimental feeding on the third day. The flank of an infected cow was shaved, and the mouth of the chimney, covered with bolting silk instead of the usual muslin, was kept firmly pressed to the shaven skin. The chimney was covered with a black cloth as an inducement to feeding, and condensation of moisture inside was prevented by the use of dehydrating crystals at the distal mouth of the chimney. It was usually necessary to keep the chimney applied to the cow for about an hour in order to obtain a reasonable number of fed flies. Even under the most favourable conditions the number of flies which took a blood meal in this period was less than half the total number in the chimney, which usually contained up to but not more than 50 flies.

The difficulties and expenditure of time involved in the breeding and feeding of such insects were so great as to render the work unprofitable, especially as the chances of infecting even one specimen with *O. gibsoni* larvae were extremely small on account of the very low infectivity rate obtained with " wild flies."

Examination of Cattle at Fraser's Hill.

In August 1936 the Government Dairy Farm at Fraser's Hill, F.M.S., was visited in order to find out if the cattle there were infected with *O. gibsoni*. The animals comprising the herd are of South African and Australian origin, and the original stock was imported into Malaya about ten years ago. It was thought that if these cattle were infected with *O. gibsoni* and it could be proved to be endemic, it would be necessary to extend the range of the investigation to this locality, where both *Simulium* and *Culicoides* occur. Accordingly a skin examination of 26 of the total herd of 47 was made but no evidence of *Onchocerca* infection was found in any of these.

Conclusions.

Although the experimental and circumstantial evidence adduced in the foregoing account is strongly in favour of the *Culicoides* theory of *O. gibsoni* transmission, its acceptance makes desirable the explanation of certain facts the work has brought forward, concerning which one can only theorise as yet. Outstanding amongst them is the extraordinarily low infection rate of the insects involved, with the larvae which are believed to be those of *O. gibsoni*. The known life-cycles of other filarial parasites, which include four species of *Onchocerca*, lead one to expect a reasonably

high infection rate in the proved insect intermediaries. The development of *O. cervicalis* in *Culicoides nubeculosus* is a possible exception. Here the experimental infection rate was shown to be as small as 5% ; but this figure, low as it is, exceeds by far the experimental infection rates found in the case of *O. gibsoni*, which in four species of *Culicoides* were less than one per cent. From a comparison of the percentage of insects in which development took place and the percentage of insects which picked up microfilariae during the infective blood meal, it is apparent, however, that the low infectivity rate is not due to an inherent unsuitability to the environment provided by the body of the insect, but to the inefficiency of the insects at picking up the microfilariae from the skin. This inefficiency is emphasised when one considers the enormous numbers of microfilariae which inhabit the skin, in a location moreover which is within easy reach of the proboscis of even the smallest species of *Culicoides*. Whatever may be the cause of this low infection rate in *Culicoides*, it does not constitute an argument, biological or otherwise, against this genus as a vector of *O. gibsoni*. The very large numbers in which these insects have been shown to congregate about and bite cattle, amply compensates for the low rate of infection in the genus as a whole. Thus, in *C. pungens* the natural infection rate is only about 3 flies per thousand ; but from the frequency in which members of this species bite cattle, there is a very high probability of a cow being bitten by at least one infected *C. pungens* once *every day*, and the same thing may be said of the species *C. oxystoma*, *C. shortti* and *C. orientalis*. There is thus no incompatibility between the high infection rate in the cattle and the low infection rate in *Culicoides*, and it may be concluded that although individual insects are inefficient, the genus *Culicoides*, taken as a whole is an efficient vector of *O. gibsoni*.

Concerning the position of the nodules, containing the adult parasites, on a fairly clearly limited region of the anatomy of infected cattle, it is natural to look for an explanation of this distribution in the biting habits of the insect vectors, for it has been suggested that the distribution of the nodules in the case of human onchocerciasis is dependent on such a factor. It has been shown however, that the biting habits of *Culicoides pungens* are not correlated with the location of the nodules of *O. gibsoni*, so that in this case the theory is not substantiated. One might also point to the case of *O. armillata*, in which it is most unlikely that the location of the

adult worm can in any way be correlated with the biting habits of its intermediate host, whatever this may be. On the evidence provided by these two cases, taken in conjunction with the analogous evidence that the infective larvae of many species of nematodes undergo a migratory phase in the body of the definitive host after gaining entry and before settling down in the habitat which is normal to the particular species, it is here suggested that the location of the different species of *Onchocerca* in the definitive host is not dependent on the biting habits of their insect vectors, but is occasioned either by a choice on the part of the parasite or by a selective action of the host, which in turn is dependent upon and varies with the particular species of parasite.

Summary.

1. The problem of the vector of *Onchocerca gibsoni* was investigated in Kuala Lumpur, F.M.S., where the parasite is endemic and a high percentage of cattle is infected.

2. For reasons which are outlined, the experimental work was confined to species of *Culicoides* and *Lasiohelea* of which 18, possibly more, species were found on cattle, some of them being found to bite cattle in very large numbers daily.

3. The distribution of the microfilariae of *O. gibsoni* in the skin of live cattle was found to be very irregular, even within small areas of skin. In transverse sections of skin the microfilariae appear to have a maximum concentration just under the epidermis at a depth of ·05 to ·2 mm.

4. 0·52% of 1,523 *Culicoides pungens*, which were dissected soon after an infective blood meal, were found to have picked up the microfilariae. 0·96% of 1,670 of this species, dissected some days after an infective blood meal, were found to have developing or mature filarial larvae in the thorax or head. 0·35% of 3,734 of this species, were found to be naturally infected with filarial larvae in the thorax or head. The difference between the experimental infection rate and the natural infection rate is shown statistically to be significant and it is concluded that *C. pungens* is an intermediate host of *O. gibsoni*.

5. *Culicoides oxystoma*, *C. shortti* and *C. orientalis* were also dissected in large numbers and were found to pick up the microfilariae of *O. gibsoni*; in each of these species the experimental infection rate exceeded the natural infection rate, but the difference in none of these is significant.

It is concluded however, from the results obtained and from the morphological similarity of the mature larvae found in these species with that in *C. pungens*, that these species are also intermediate hosts of *O. gibsoni*.

6. *C. peregrinus* and *C. buckleyi* are suspected but not proven intermediate hosts. *C. anophelis* and/or *C. anophelis* v. *flavescens* and *Lasiohelea stimulans* were found to pick up the microfilariae but only partial development was found in them. *C. sumatrae* and *C. raripalpis* also picked up the microfilariae but no development was found in them. The remaining species worked with were entirely negative. In view, however, of the very minute percentage of infection which occurs in *Culicoides* spp. and the large numbers which must be dissected to obtain positive results, the negative results given by these less common species are not necessarily conclusive.

Acknowledgments.

The writer has much pleasure in acknowledging his indebtedness to Dr. A. Neave Kingsbury, Director of the Institute for Medical Research, F.M.S., for his courtesy in providing laboratory accommodation and facilities for the investigation; to Capt. S. H. Whitworth, Director of Veterinary Research and Veterinary Adviser, Malaya, for his interest in the investigation and the freely-offered facilities of the Veterinary Department which were gratefully accepted; to Mr. E. P. Hodgkin, B.Sc., of the Institute for Medical Research for his generous co-operation and advice in many matters appertaining to the entomological aspects of the work and to his assistant Mr. A. G. Pilai; to Mr. R. M. Devar, owner of the Kuala Lumpur Dairy Farm; to Dr. J. O. Irwin for help with the statistics; to Dr. J. W. S. Macfie and Dr. F. W. Edwards for the identification of the insects; to Dr. F. E. Byron for the photomicrographs of *Culicoides* wings; and to Mr. W. A. McDonald for the other photomicrographs. The work was carried out under the direction of Professor R. T. Leiper, F.R.S., whose guidance and encouragement were throughout an essential part of the investigation.

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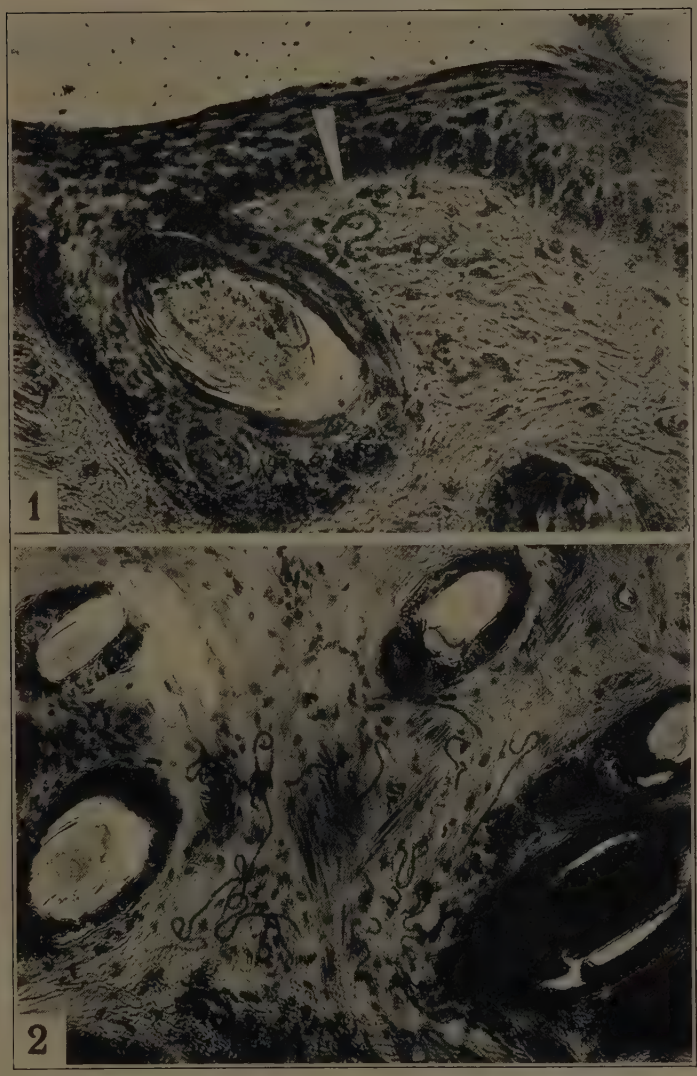

Fig. 1.—Transverse section of skin of cow showing a microfilaria of *O. gibsoni* in the dermis about ·05 mm. from the skin surface. ×400. Fig. 2.—Tangential section of skin of cow showing *O. gibsoni* microfilariae at a depth of about ·1 mm. from the skin surface. ×180.

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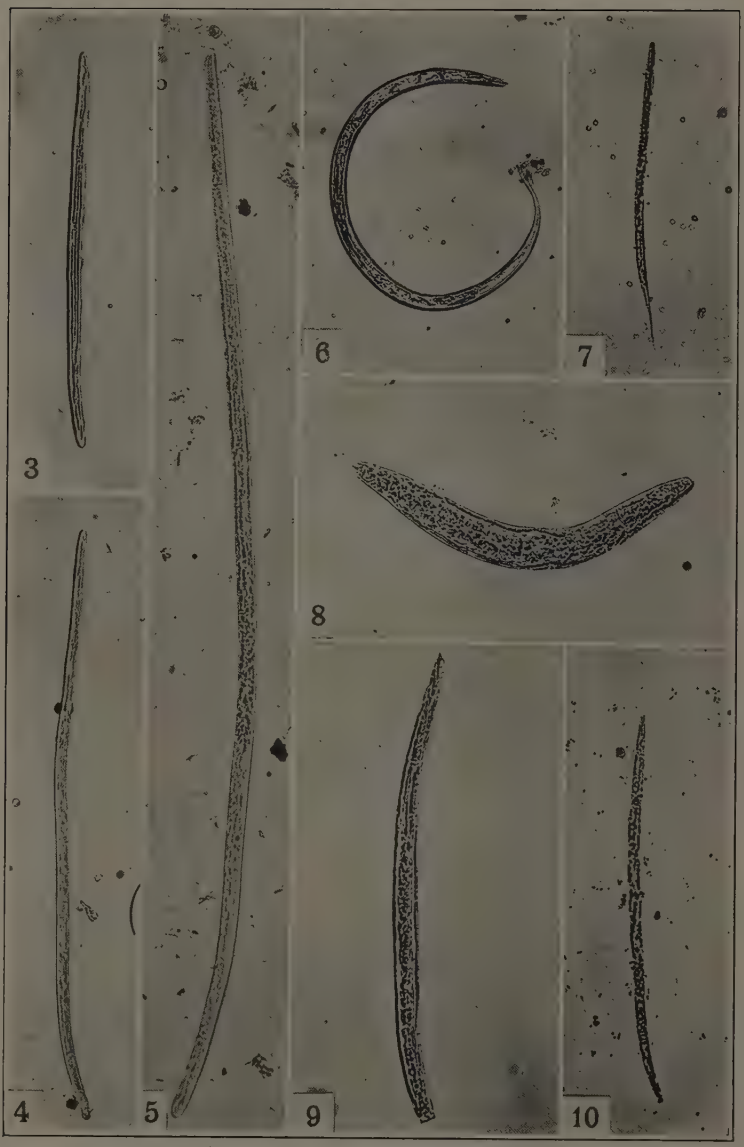


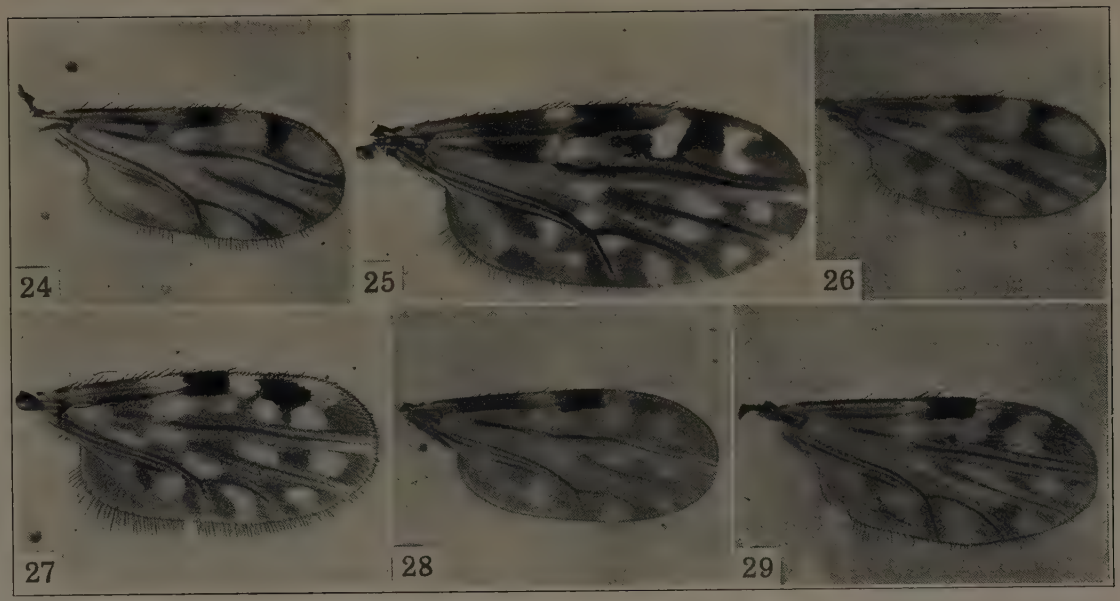
Fig. 3.—Type A filarial larva. Fig. 4.—Type B filarial larva. Fig. 5.—Type C filarial larva. Figs. 6–10.—Non-filarial nematode larvae from *Culicoides*. Figs. 3–10. ×145.

PLATE III.

Fig. 11.—Microfilaria of *O. gibsoni* from the stomach of *C. pungens* dissected a few hours after an infective blood meal. Figs. 12–17.—Developing stages dissected from *C. pungens* and *C. oxystoma*. Fig. 18.—Mature larva from the head of *C. pungens*. Fig. 19.—Two mature larvae partly dissected from the head of *C. oxystoma*. Figs. 11–19. ×145.

Fig. 20.—Collecting *Culicoides* from a cow by the test-tube method. Fig. 21.—Artificial feeding of *Culicoides* on an infected cow. Fig. 22.—Collecting breeding material (for *C. oxystoma* and *C. peregrinus*) from the margins of a stream. Fig. 23.—Breeding places of *C. oxystoma* and *C. peregrinus* in a drain (foreground) and of *C. orientalis* and *C. peregrinus* on an old manure heap near cattle pen (background).

PLATE V.



Wings of *Culicoides*. Fig. 24.—*C. pungens*. Fig. 25.—*C. peregrinus*. Fig. 26.—*C. orientalis*. Fig. 27.—*C. oxystoma*. Fig. 28.—*C. buckleyi*. Fig. 29.—*C. shortti*. Figs. 24–29. ×60.

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Observations on the Destruction of the Stem Eelworm, *Anguillulina dipsaci*, by the Fungus *Arthrobotrys oligospora* Fres.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

Introduction.

Fungi, which entrap and destroy small nematodes, have been known to mycologists for many years. Drechsler, who, for a long time, has made a special study of these forms, has recently, in an important paper (1937), described and figured several species, including many new ones belonging to a number of different genera.

Amongst these fungi is one, *Arthrobotrys oligospora* Fresenius, which has long been known from the investigations of Zopf (1888) for its ability to capture, destroy and feed upon small free-living nematodes and also upon the larvae of *Anguillulina tritici* when supplied to it under experimental conditions. The fungus is of widespread occurrence in soil, on the dung of various animals and other media rich in decaying organic matter. Drechsler (l.c.) p. 464, says " After addition of small masses of decomposing refuse to nematode-infested agar plate cultures, it makes its appearance not only more frequently than any other of the related predacious forms, but perhaps in larger quantity than all the other predacious forms taken together." He also directs attention to various papers in which the nematode-capturing habits of the fungus are reported and substantiated. Amongst these may be cited Rahm (1922) where it is stated that nematodes belonging to the genera *Tylenchus* and *Mononchus*, normally living among mosses, became entrapped by the hyphal loops of this fungus and were finally destroyed by it. Sherbakoff (1933) mentions the occurrence of *A. oligospora* on agar plate cultures in association with another nematode-capturing species *Anulosporium nematogenum*. Linford (1937) also has recently reported the presence of *A. oligospora* in Hawaiian soils along

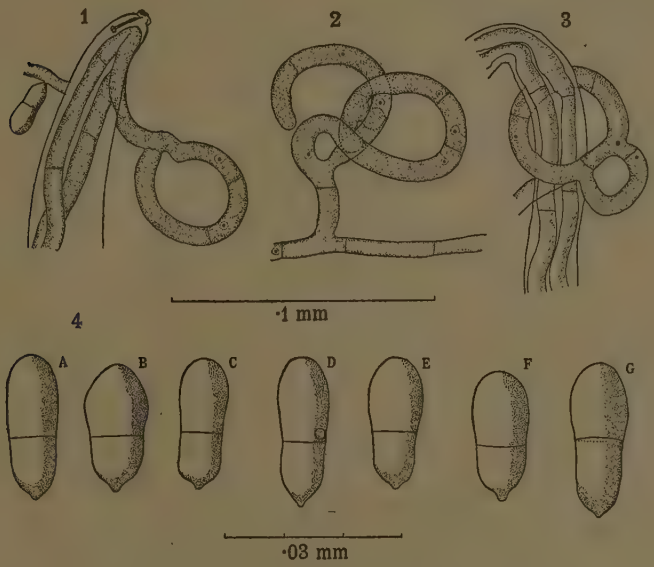
with a number of other known species of nematode-destroying fungi and several which appear to be previously undescribed forms. He stresses the importance of such fungi in the destruction of the larvae of *Heterodera marioni* in soil rich in organic matter.

The predacious powers of *A. oligospora* and similar fungi are well authenticated and Linford's observations show how important such behaviour may be in the biological relationships normally pertaining in soils, particularly those containing an abundance of organic matter. As far as the writer is aware, however, there do not appear to be any records of such fungi invading plants and there destroying plant parasitic nematodes whilst these are actually infesting the tissues of the host. The purpose of the present note is to put on record observations based on two such occurrences where *A. oligospora* was found capturing and destroying *Anguillulina dipsaci* within plant tissues. One of these was in 1938 and the other in 1928. The more recent observations are more detailed and thorough than the earlier ones and are dealt with first.

Plant Hosts.

1. *Calceolaria integrifolia* L. (yellow calceolaria).—Towards the end of March, 1938, 4 small plants of yellow calceolaria, variety "Little Gem," were sent to the writer for examination by Mr. S. G. Jary of the South-Eastern Agricultural College, Wye, Kent. The plants were from 2–3 inches high and many of the leaves showed considerable areas of brownish-black discoloration with some swelling of the tissues in the vicinity of the main vein on the underside of the leaf-stalk. (The disease symptoms were essentially similar to those found by the writer (1929) on the leaves of yellow calceolaria plants which were sent from the Manchester area by Dr. H. W. Miles.) On teasing up some of the blackened tissues in water it was found that they were heavily parasitized by the stem eelworm, *Anguillulina dipsaci*. A white fungal mycelium was also present along with the nematode parasites and it was soon apparent, even under low magnification, that large numbers of the worms were entangled in the fungal hyphae. On examining preparations under higher magnification it became clear that many worms were completely parasitized by the fungus, hyphae of which could be seen within their bodies. Numbers of worms, also, were seen to be struggling fruitlessly in efforts to extricate themselves from the hyphae. Some were found with one or more constricting rings or more complicated hyphal loops

tightly encircling the body. Such anastomosing loops were also plentiful on many of the hyphae and it was clear that the fungus was one of those in which such structures serve as snares for nematodes. The loops



Anguillulina dipsaci attacked by *Arthrobotrys oligospora.*

Fig. 1.—Anterior end of *A. dipsaci* showing body invaded by hyphae of *A. oligospora*; mouth spear displaced by hyphae; ensnaring loop on right side of body and a single conidia on left side.

Fig. 2.—A double ensnaring loop of *A. oligospora* showing anastomosis of hyphae in centre.

Fig. 3.—Part of body of *A. dipsaci* larva within a loop of *A. oligospora.* Figs. 1, 2 and 3 all drawn to the same scale.

Fig. 4,A—G conidia of *A. oligospora* drawn under high magnification. A—F from *Calceolaria integrifolia*, G from *Saxifraga Cotyledon.*

(figs. 1, 2 and 3) as well as the pyriform, uni-septate conidia, which were also found (fig. 4) agreed well in shape and size with those of *Arthrobotrys oligospora* Fres., as described and figured by Drechsler (1937). The decaying plant parts were kept in dishes in a moist atmosphere and after a few days an abundant crop of downy white sporophores, with heads of pyriform conidia attached, made its appearance on the tissues. These

fruiting bodies closely resembled those described by Drechsler for *A. oligospora*. Some of the conidia borne by them were mounted and drawn under high magnification and were found to agree in shape and size with the conidia of *A. oligospora*; being from 22 to 25μ long by 10 to 12μ in greatest width.

2. *Saxifraga Cotyledon*, L.—A plant of this species, sent to the writer by Mr. G. Fox Wilson in November, 1928, from the Gardens of the Royal Horticultural Society at Wisley, Surrey, showed a rot of the crown with blackening of the leaves. *Anguillulina dipsaci* was found in the diseased tissues and many of the worms were entangled in fungal hyphae. Numbers of them were dead and filled with hyphae and here and there pyriform conidia were observed on short hyphal branches sticking out from the surface of the cuticle. The diseased leaf material was left in a shallow layer of water in a Petri dish for a few days, by which time a few sporophores with heads of pyriform conidia had pushed up above the surface of the water. A freehand sketch was made of one of these structures bearing two heads, one below the other, and on comparing this with Drechsler's illustrations of these bodies it shows good agreement in appearance with the sporophore of *Arthrobotrys* species. At the same time a camera lucida drawing of one of the pyriform conidia was also made under high magnification and on comparing this with the same author's figures of the conidia of *A. oligospora* the two are found to agree in shape and size. There can be no doubt, therefore, that in the case of this host the fungus concerned in the capture and destruction of the eelworms was *Arthrobotrys oligospora*. Unfortunately, the writer's notes and drawings made at the time the observations were carried out make no mention of his having seen the ensnaring loops and rings. The sporophore and conidia, however, have revealed the identity of the fungus.

Discussion.

Earlier workers have shown that the fungus, *Arthrobotrys oligospora*, occurs in soil and in other media containing decomposing organic matter and that it effectively destroys small nematodes which it ensnares by means of special organs of capture. The writer's observations extend our knowledge of the sphere of activity of the fungus since they show that it can capture and destroy the stem eelworm, *Anguillulina dipsaci*, that it is

capable of entering the tissues of host plants and of there bringing about the destruction of large numbers of the parasite. How the fungus invades the tissues of the host plant is not apparent but it must clearly be from the soil. It is scarcely likely that individual eelworms already parasitized by the fungus would be the means of its introduction since such worms are quickly killed by the growth of the fungus within the body and are, moreover, so rapidly immobilized that they would be incapable of migratory movement. On the whole it seems probable that the fungus reaches the plant either by the spread of fungal hyphae into the tissues from the soil or by means of conidia which might be splashed on to it from the soil during watering. Those regions of the stem, at or just below soil level, already parasitized by the eelworm could readily become invaded by hyphae. Conidia making a lodgement on such regions would, no doubt, germinate and produce hyphae which would grow on the tissues already diseased as a result of eelworm attack. In this medium of decaying plant material, together with the abundant supply of eelworms as a source of food, the fungus would grow and spread throughout those areas primarily attacked by the eelworm.

It is interesting to speculate whether in this manner the fungus can exercise some measure of biological control over the parasite and so check its further spread in the host's tissues. The writer's observations are on too limited a scale for him to state positively that disease caused by the eelworm is checked by this predacious fungus. All that he can say is that large numbers of the nematode are destroyed by it and since, as a consequence, the numbers of the nematode eventually reaching the soil from the diseased plant must be considerably reduced it may be claimed that the fungus ultimately exerts some control over the parasite by checking its multiplication and further spread.

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Observations on the Helminth Parasites of Poultry in Scotland.

By D. O. MORGAN, M.Sc., Ph.D.
(*Lecturer in Helminthology, the University of Edinburgh and The Royal (Dick) Veterinary College.*)

and J. E. WILSON, B.Sc., M.R.C.V.S.
(*Department of Poultry Diseases, Royal (Dick) Veterinary College.*)

THE increasing mortality among domestic fowls in this country has of recent years drawn attention to the possibility that worms and other parasites may be factors in the causation of certain poultry diseases. Heavy infestations with worms are frequently observed on farms where mortality is high and although it is rare that death can be directly attributed to the parasites, it is thought that they might be responsible for loss of condition in the birds, and for lowering their resistance to disease. On the other hand, equally heavy infestations may be found in apparently healthy birds and the few experiments which have been carried out to test the pathogenicity of certain common intestinal worms have not shown any marked effect on the health of the birds as a result of the infestation. Further work along these lines is however, necessary before the pathogenicity of helminths in poultry and their relation to disease is understood.

The observations recorded in this paper were made during a survey of the helminth parasites of diseased poultry sent from various parts of Scotland to the Royal (Dick) Veterinary College, Edinburgh, for post-mortem examination. The object of the survey was to find out the incidence of parasitic worms in diseased birds and what species were commonly present in numbers likely to influence the health of the birds. It was also thought that the survey might throw some light on the possible relationship between worms and certain specific diseases.

The survey was commenced in October 1933 and continued over a period of two years. During that time 1,113 adult birds ranging in age from about 6 months to 2 years were examined.

Technique.

The intestine of each bird was opened and the contents sedimented a few times in water and afterwards examined in a shallow dish, the caecal contents being kept separate from those of the remainder of the intestine. Only in the case of *Heterakis gallinae* and *Ascaridia galli* were actual counts made as it was found that this involved too much time for some of the other species. *Davainea proglottina*, in particular, presented difficulties, owing to the fact that disintegration had usually set in when the birds were received and complete specimens of this species were rarely obtained. For these species, therefore, it was decided to designate the level of infection as " light," " medium " or " heavy," and a few actual counts of *Capillaria* spp. showed that an infection referred to as " light " would indicate from 1 to 100 worms ; " medium " from 100 to 300 and " heavy " 300 and upwards.

Results of Survey.

The survey showed that very few of the species recorded from the domestic fowl occur in this host in Scotland, and that a still fewer number have a high incidence. The following species were found :—*Heterakis gallinae*, *Ascaridia galli*, *Capillaria longicollis*, *C. columbae*, *C. retusa*, *Davainea proglottina*, *Trichostrongylus tenuis*, *Raillietina cesticillus*, *Amoebotaenia sphenoides* and *Hymenolepis carioca*. Of the trematodes only one macerated specimen was obtained and this was tentatively referred to the genus *Plagiorchis*.

Heterakis gallinae. This species was by far the most common and 82·3% of the birds examined were found to be infected, with an average of 99·1 worms per bird. The number of worms present in individual birds varied considerably and anything from 1 to over 1,000 were found, while 26·4% of the infected birds harboured over 100 worms. In view of the difficulty encountered in producing heavy infections with this species under experimental conditions (Clapham 1934) these figures are of interest since 300 to 500 worms are very commonly met with in naturally infected birds. It was thought that the heavy infections of *Heterakis gallinae* might be attributed to the fact that the birds examined were diseased

and that the resistance of the birds to this species might thus be lowered. That this was not the case was found when a number of *healthy* birds were examined and shown to harbour infections which were quite as heavy as those in diseased birds.

As regards seasonal incidence, there appeared to be no marked variation in the percentage infected with *Heterakis gallinae*, except for a slight fall in the early months of the year and a rise in late autumn. The percentage infected during the period from January to March was 77·2 and from October to December 90·5. Similarly, of the infected birds a smaller percentage (15·8) had over 100 worms in the first quarter of the year than in the last quarter when the figure obtained was 30·4%. By far the lowest incidence was obtained in February both in 1934 and 1935, when only one bird out of a total of 76 harboured over 100 worms.

Capillaria spp. As already stated, three species of this genus were found, but only two of these, *C. longicollis* and *C. columbae* occurred with any frequency. *C. retusa* was relatively uncommon, a finding in accord with previous observations (Morgan 1933) on *Capillaria* species in fowls. In the birds examined in this survey one or more of the species of *Capillaria* were found in 43·0% and, with the exception of *Heterakis gallinae*, are undoubtedly the most common worms occurring in fowls in this country. *C. longicollis* was found in 36·7% of the cases; *C. columbae* in 8·4% and *C. retusa* in 4·6%. The percentage figure given for *C. columbae* is probably too low as this species frequently occurs in association with *C. longicollis* in the small intestine and where a large number of worms are present a close study of each specimen has to be made before its presence or absence can be stated with certainty. As this could not be carried out where infections were heavy, it is thought probable that a few light infections with *C. columbae* were missed.

Of the infected birds, 29·4% harboured heavy infections, chiefly with *C. longicollis*, while no heavy infections with *C. retusa* were found, the number of worms present in the case of the latter species being usually less than 10. Out of 94 cases infected with *C. columbae*, 24 were heavily infected.

As in the case of *Heterakis gallinae*, the incidence of *Capillaria* spp. was found to be low in the early months of the year (22·8%) but was highest during the period April to June (54·3%) although a very high incidence was also obtained in the month of October (58·1%). The

month of April followed closely with 57·7%. Heavy infections were rare in January and February and most frequent from May onwards.

Davainea proglottina. Of the numerous cestodes which have been described from the domestic fowl this is the only species which occurs with any frequency in this country. The worm was found in 14·9% of the birds examined, but it is probable that this figure is a little on the low side as very light infections can easily be missed because the worm is very small and the segments very soon break up. As in the case of the previous species, the incidence was low in January and February and high from June to December, while heavy infections were also more frequently met with during the latter period.

Ascaridia galli. This species occurred in only 11·1% of the birds examined and no marked seasonal variation was observed. The number of worms found in each bird was usually very small and out of 123 infected birds only 6 harboured more than 10 worms. In one bird the exceptionally large number of 96 worms was found, many of which had migrated into the peritoneal cavity, although no perforation of the intestine could be found. In this case death was directly attributed to the worm invasion. Even including this exceptional case, the average number of worms per bird was slightly less than 5.

As all the birds examined were over six months old, the light infection usually observed tends to confirm the view that an immunity is established against *Ascaridia galli* when fowls are about three months old (Ackert & Herrick 1928).

Trichostrongylus tenuis. Although this species is particularly common in grouse and partridges in Scotland, its occurrence in the domestic fowl is comparatively rare. The species was only found on 12 occasions in the survey and with one exception, when 22 worms were present, infections usually consisted of one or two specimens. It seems, therefore, that fowls are partially immune to *T. tenuis* and that infections in this host do not normally reach a level when disease symptoms are produced. In game birds, on the other hand, the species appears to be particularly pathogenic.

Other species. As already mentioned, apart from *Davainea proglottina*, cestodes were of rare occurrence. *Raillietina cesticillus* was found in 7 of the birds, *Amoebotaenia sphenoides* in 2, and in 2 others, young specimens of what appeared to be *Hymenolepis carioca.*

Remarks on Incidence of Species.

It will be seen from the records given above that very few species are common in poultry in Scotland and assuming that all worms are pathogenic when present in large numbers, the only species likely to be causing widespread damage are *Heterakis gallinae*, *Capillaria* spp. (chiefly *C. longicollis*) and *Davainea proglottina*. *Ascaridia galli* should perhaps be included in this list, as it may be responsible for considerable damage among young birds up to the age of three months. The survey showed that old birds are, at least, important as carriers of this species and in one instance it was the cause of death.

Worms and Disease.

As already stated, all the birds examined during the survey had died from one or other of the diseases common to poultry in Scotland. A large proportion had suffered from fowl paralysis and since worms have been suggested as one of the causes of this disease, it was thought that a comparison between the incidence of heavy infections in fowl paralysis cases and that in birds suffering from other diseases, as well as in healthy birds, might throw some light on this problem.

The 388 cases of fowl paralysis examined showed 28·1 per cent. to be heavily infected with worms, with 14·1% entirely free from worms. In 480 cases suffering from diseases other than fowl paralysis and coccidiosis, 26·3% had heavy worm infections and 11·0% were entirely free from worms. It will be seen that the difference in the incidence of heavy infestations in these two groups is too small to suggest any association between worms and fowl paralysis.

A comparison was also made with a number of birds killed for market and which were apparently in good health. These birds were obtained from a poultry farm where worms were known to be common but where fowl paralysis was practically non-existent. The result of the examination showed that these healthy birds from this particular poultry farm (Farm A) had a higher incidence of infection as well as a higher percentage of " heavily infected " than was found in the diseased birds of the general survey.

For example, the figures for *Heterakis gallinae*, the most common of all the worms in poultry, were as follows :—

	Healthy birds.	Diseased birds.
Percentage infected	97·1	82·3
Percentage with over 100 worms	46·7	26·4

Similarly with the other species, the worms were more common in the healthy than in the diseased birds.

A further comparison was made with the results obtained from the examination of 285 birds obtained from another poultry farm (Farm B) where the incidence of disease, particularly fowl paralysis, was very high. These results are shown in the following table together with those obtained from Farm A (consisting of healthy birds) and those from the general survey :—

	Farm A Healthy birds	Farm B Diseased birds	General Survey Diseased birds
% heavily infected with one or more species of worms	41·2	15·4	22·4
% infected with *H. gallinae*	97·1	73·0	82·3
% of *H. gallinae* infected birds with over 100 worms	46·7	12·0	26·4
% infected with *Capillaria* spp....	50·0	69·1	43·0
% infected with *D. proglottina*	28·8	17·9	14·9
% infected with *A. galli*	30·6	9·8	11·1

It will be seen from the above results that the incidence and the intensity of the infections with worms are in almost all instances higher in the healthy birds from Farm A than they are in the diseased birds from the other two sources. The only exception is the lower incidence of *Capillaria* in Farm A than in Farm B, but even here the figure is higher than that obtained in the diseased birds of the general survey.

It is realised, however, that such a distinction would probably not be obtained by an examination of healthy birds from other farms as the prevalence of worms depends to some extent on the intensity of the

infestation of worm eggs on the runs, which in turn depends on the number of years such ground has carried poultry and the extent of the stocking. Farm A is nevertheless of particular interest in view of the suggestion that worms may be associated with fowl paralysis. This disease was exceedingly rare on this farm, although it had been heavily stocked for many years and showed a high level of infestations with both worms and coccidia.

Apart from fowl paralysis, none of the other diseases was obtained in a sufficient number of birds for comparisons to be made between their worm burden and that of fowl paralysis cases. An exception might be made in the case of coccidiosis, which was diagnosed as of major importance in 155 birds but in a large proportion of these fowl paralysis was also observed. These cases did, however, show 37% with heavy worm infestations, a percentage which is higher than that given for any group except the healthy birds.

The 53 cases of tuberculosis which were obtained during the survey were of particular interest in that worms were very often absent or present in small numbers. Only 6 of these birds could be said to be even moderately heavily infected. Subsequent studies on birds from other sources have confirmed this observation that tuberculosis is associated with a markedly low level of worm infestation in fowls and where a fair number of worms occur the disease is invariably at an early stage.

In a recent paper (1938) Clapham has brought forward evidence to show that fowls suffering from leukaemia have a lower resistance to helminthic infestations than healthy birds. Only 18 birds suffering from this disease were obtained during the general survey, and the number is therefore too small for comparisons to be made with other diseases or with healthy birds. There appeared to be nothing exceptional in the infestations of these 18 birds and only 5 of them harboured large numbers of worms while 3 were entirely free from infestation. It should be pointed out, however, that while Clapham's observations were made on birds from one farm, those examined in the general survey were drawn from different centres in Scotland and the chances of picking up helminth eggs and larvae would therefore vary very considerably.

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The Longevity of *Fasciola hepatica.*

By J. W. G. Leiper, M.R.C.V.S.

(*Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

There appears to be a common belief among veterinarians that liver-fluke infections, caused by *Fasciola hepatica*, are only transient and that if the animals do not succumb to the disease the parasites pass out of the body in about nine months' time. For instance, Mönnig's " Veterinary Helminthology and Entomology " states on p. 39 : " The flukes (*Fasciola hepatica*) usually live about 9 months in the sheep and then die and pass out through the intestine." Clunies Ross & Gordon, in their " Parasites of Sheep in Australia " (1936), say : " The majority of flukes probably die after about 9 months but some at least may survive for 2 years." One might question on what scientific evidence these statements are based. Thomas (1881), discussing longevity, says with regard to one sheep examined : " The ewe had been kept under conditions which render re-infection highly improbable, and it follows, therefore, that *the life of the liver-fluke may extend beyond one year.*"

Hutyra & Marek (1920, 2nd English edition), state : " According to Gerlach the period spent by the distomes in the liver is from 9 to 12 months." In their 3rd English edition (1926), this is replaced by a more extensive paragraph : " The flukes remain in the liver for a period of from 3 to 5 years. Railliet, Moussu & Henry (1913) found them after 3 years and Thomas after 5¼ years in animals that had no opportunity to become re-infected during this period. The majority of the flukes however leave the liver in from 9 months to 1 year . . ." The present writer, however, can find no reference in Thomas's work to longevity of the parasite beyond 13 months.

As far as can be ascertained, the longest life of *Fasciola hepatica* authentically recorded in sheep, is given by Railliet, Moussu & Henry who state : " Quant à la durée de la vie de ces parasites, elle peut dépasser aussi de beaucoup les limites généralement acceptées. On a prétendu que les Douves installées dans le foie vers l'automme en sont

régulièrement expulsées en mai ou juin ; or, nous en avons trouvé, à Alfort, dans les canaux biliaires d'un mouton isolé et soustrait aux conditions d'infestation pendant près de trois ans."

Montgomerie (1931) recorded the longevity of *Fasciola hepatica* in three rabbits experimentally infected, and kept under conditions where there was no possibility of natural infection. Flukes were recovered at post-mortem 13 months, 2 years 7 months, and 3 years 1 month after infection.

The experiments about to be described show that, in goats at any rate, large numbers of *Fasciola hepatica* have lived and are still alive and laying fertile eggs 4 years 9 months and 12 days after infection.

Sixteen goats, bred and reared at the Institute of Agricultural Parasitology, were infected, by Professor R. T. Leiper in 1933, with encysted cercariae of *Fasciola hepatica*.

The cercariae were obtained by culturing eggs from the gall bladders of abattoir cattle and infecting *Limnaea truncatula* with the miracidia. Fifteen goats were given from 30 to 100 cercariae each on the 17th September, 1933, and one goat 100 cercariae on the 21st December, 1933. These sixteen goats were all about 6 months old.

The goats were housed during the winter, and in the summer were placed on pasture where there was no chance of natural re-infection occurring. Numerous post-mortem examinations of sheep and goats grazed on the farm have been carried out but in no case has a naturally acquired infection with *Fasciola hepatica* been found.

The dose given was not intended to cause serious symptoms and the animals were found to tolerate the infection well. They were, however, not as healthy as the stock animals. *Fasciola* eggs were first found in the faeces 78 days after infection, *i.e.*, about 11 weeks. Since the commencement of the experiment eleven goats have died or were killed. The remaining five goats were all still positive on 29th June, 1938.

The cause of death in the majority of cases was gastro-intestinal helminthiasis brought about by the banking up of infection on the pasture where the goats were placed each summer. The total numbers of helminths recovered were between 46,500 and 8,100 worms, the great majority being *Trichostrongylus axei*, *T. colubriformis*, and *Osterlagia circumcincta*, but *Haemonchus contortus*, *Oesophagostomum venulosum*, *Chabertia ovina*, *Trichostrongylus vitrinus* and *T. capricola* were also found to be present.

The accompanying table shows the numbers of encysted cercariae fed and the numbers of *Fasciola hepatica* recovered. It will be noticed that a high percentage has been recovered after the varying period of life of the infected host.

Goats infected experimentally on one occasion only with *Fasciola hepatica*.

Goat	Date of feeding	No. of encysted cercariae given	Date of death	Cause of Death	*F. hepatica* recovered at post-mortem	
					No.	Percentage
F.1	17/9/33	50	29/6/34	Shot	12	24
F.3	,,	100	14/10/36	Gastro-intestinal helminthiasis	29	29
F.5	,,	50	1/5/34	Shot	24	48
F.7	,,	60	20/9/36	Gastro-intestinal helminthiasis	6 (in part of liver)	—
F.12	,,	50	9/4/36	,, ,,	7 at least	—
F.15	,,	60	2/6/35	,, ,,	9	15
F.17	,,	50	29/11/36	,, ,,	13	26
F.19	,,	50	15/9/36	,, ,,	12	24
F.22	25/11/33	100	26/4/38	Shot	56	56
F.23	17/9/33	60	25/11/36	Gastro-intestinal helminthiasis	18	30
F.27	,,	50	8/10/36	,, ,,	29	58
F.2	,,	30	Alive 29/6/38	Alive	Eggs in faeces	
F.4	,,	50				
F.20	,,	50				
F.24	,,	50				
F.28	,,	50				
					Average	35·5%

It appears from the above data that the goat has not the power of throwing off its parasites, after 9 months or so, as has been suggested for sheep. The fluke can live for many years in goats and if this is also true for sheep this is an explanation for the sudden appearance of liver rot in sheep immediately following a prolific year for the snail. One year of

adverse weather for *Limnaea truncatula* would, one would suppose, lead to a great reduction of adult flukes in the following year if their life was only 9 months or so, and it would take some years before large infections were again available to the definitive host.

The matter of longevity is important (i) directly to the host, where there are not only the local reactions causing fibrosis of the liver but also systemic disturbances due to the presence of the flukes for a period of five years or more ; (ii) from the point of view of control : to rid an area of fluke would necessitate freeing it from *Limnaea truncatula* for the length of time infection remains in the infected animals, which may be, as far as we can judge at present, as long as the life of its host.

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Experiments to determine the relative Toxicity of Ammonium Chloro-acetate and related Chemicals to the Potato Eelworm (*Heterodera schachtii*).

By Enid M. Smedley, M.Sc.

(*from the Department of Helminthology, London School of Hygiene and Tropical Medicine*).

Since 1935 a large number of chemicals including chloro-acetic acid and various water-soluble chloro-acetates, received from the Pest Control Committee of Imperial Chemical Industries, have been tested for their value in controlling the potato strain of *Heterodera schachtii* under the direction of Prof. R. T. Leiper, F.R.S.

Soils from Yorkshire and Ayrshire were used in the experiments, as they are of very different types, and might conceivably respond differently to chemical treatment.

The soil from Yorkshire is a very fine silt. It "pans" when wet, forming very hard lumps when dried. The cysts are clean and well-filled but not numerous, averaging about 40 per 50 g. of soil. By contrast the Ayrshire soil is almost a pure sand. Although the cyst content is very high, about 300 per 50 g. sample, they are often poorly filled. There is also a great deal of undecomposed plant material.

The chemicals were first tested for their direct action on the parasites. Freshly hatched larvae of *H. schachtii* were placed in solutions or suspensions of each chemical and when apparently dead were removed to clean tap water to allow of any recovery. The larvae survived immersion in 1% calcium acetate, calcium chloride or calcium glycollate for 48 hours or longer. 15 hours exposure to 1% ammonium chloro-acetate, ethyl chloro-acetate, chloro-acetic acid or acetic acid, or to 0·5% ferric chloro-acetate or lime, killed them. When exposed to the action of 0·1% ammonium chloro-acetate for the same time a percentage of the larvae appeared not to have been killed, but these failed to recover in water.

The action of the chemicals on cysts in soil was then tested experimentally. 100 g. samples of infected Ayrshire and Yorkshire soils were used, each of the nine chemicals being applied at the rate of one ton per acre, *i.e.*, 0·1% by weight of dry soil. Each chemical was thoroughly stirred into a soil sample and allowed to stand in a warm room for three weeks, during which time it was kept moist. The samples were then air-dried, and 50 g. of soil was removed from each. The cysts were washed out by the usual method, counted and placed in fresh root excretion.

The hatching results from these tests are set out in Table I. It will be noticed that the rate of hatching is much lower in the Ayrshire cysts—a constant feature, and presumably correlated with the fact that they are not well-filled, as noted above. It has been suggested in explanation of the latter that the early lifting of the potato crop in this part of Scotland constitutes in fact a kind of " trap cropping."

TABLE I.

Chemical.	Ayrshire Soil.		Yorkshire Soil.	
	Numbers of cysts.	Hatched larvae.	Numbers of cysts.	Hatched larvae.
Chloro-acetic acid (mono)	375	84	38	93
Ammonium chloro-acetate ...	339	0*	39	4*
Calcium chloro-acetate	269	65	43	144
Ferric chloro-acetate	329	44	62	4*
Ethyl chloro-acetate	347	29	45	418
Calcium acetate	259	229	40	72
Calcium chloride	359	484	46	258
Calcium glycollate	287	108	64	512
Lime	300	183	37	253
Acetic acid	194	0*	41	236

The figures in Table I represent the totals of eight weeks' hatching from October to December. The dishes were then replenished with fresh leachings, when renewed activity was noted except in four dishes. These are marked in the table by an asterisk. Dissection of the cysts in these

four experiments showed that in the case of the two soil samples treated with ammonium chloro-acetate the chemical had killed the cysts, whereas the other two dishes had been invaded by moulds which were probably responsible for the death of the parasites.

A similar experiment was then set up to find the minimum effective dose of ammonium chloro-acetate. 100 g. samples of Ayrshire and of Yorkshire soils were again used for each test, the applications varying in strength from one cwt. to one ton per acre. To avoid inaccuracy in weighing such small amounts (·005–0·1 g.) a charcoal mixture was prepared containing only 10% of the chemical by weight. The doses were then weighed as 0·05–1·0 g., and each thoroughly mixed with a soil

TABLE II.

Treatment.	Ayrshire Soil.		Yorkshire Soil.	
	Numbers of cysts.	Hatched larvae.	Numbers of cysts.	Hatched larvae.
Control (no treatment) ...	651	531	69	about 2,000
1 cwt. per acre	701	773	75	„ 2,000
2 „ „ „	681	several thousands	53	„ 2,000
5 „ „ „	740	49	72	445
10 „ „ „	823	0	68	9
15 „ „ „	566	0	64	0
20 „ „ „	802	0	66	0

sample. These were moistened and kept in covered Petri dishes for two weeks. They were then air-dried and the cysts washed out and counted as in the previous test. The cyst counts from each of the 100 g. samples, together with the results of the hatching test in fresh root excretion, are set out in Table II: the figures are for two months from December to February. Apparently in the Yorkshire soil an application of 15 cwt. per acre is necessary to control the parasite completely, although in the Ayrshire soil the pest appears to be killed by only 10 cwt.

Conclusions.

Laboratory tests indicate that various chloro-acetates have a toxic effect on the larvae and cysts of *Heterodera schachtii* and that ammonium chloro-acetate especially should effect good control of the potato strain of *Heterodera schachtii* at practicable strengths as 15 cwts. per acre in Yorkshire soil, or 10 cwts. per acre in Ayrshire soil gives complete control, while the majority of the cysts are killed by applications of 10 cwts. and 5 cwts., respectively. It is anticipated that treatment with the higher concentrations will be necessary to achieve control in the field, because of the inefficiency of our present methods of incorporating chemicals with the soil.

Acknowledgments.

The Institute of Agricultural Parasitology was indebted to Mr. A. E. Godfrey for a quantity of infected potato soil from the Yorkshire area and to Dr. D. G. O'Brien of the West of Scotland Agricultural College who very kindly provided a similar quantity of infected soil from the Ayrshire potato-growing area. Samples of these were used in the experiments now recorded.

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Biometrical Observations on Shells of *Limnaea* Species.

By B. G. PETERS, M.Sc., Ph.D.

(*A Principal Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

INTRODUCTION.

AIDED by a grant from the Agricultural Research Council, the author has undertaken an investigation into the ecology of *Limnaea truncatula*, the vector of *Fasciola hepatica* in Britain. One of the first problems facing anyone who deals with this gastropod is that of correctly differentiating it from other freshwater snails which resemble it. The tyro quickly learns to identify a typical specimen from a careful inspection of the shell ; he notes its general shape and size, the fact that it is dextrally coiled, the relative size of the shell mouth, and above all the comparatively deep suture which runs between adjacent coils of the spiral. If the shell contains an active snail, attention is drawn to the dark gray colour of the exposed part of the body, and to the single pair of short, conical tentacles characteristic of all *Limnaea* species.

But, unfortunately, not all specimens of *L. truncatula* are " typical " : there is considerable variation between local races. There is also much variation in the related species of *Limnaea*, of which *L. palustris* in its young stages often closely resembles it, so that confusion is liable to occur. Reichmuth (1936) has shown that there are reliable anatomical differences between these two species but, for the helminthologist especially, it is desirable to be able to identify them without killing them. With this end in view, various dimensions and ratios have been determined for shells of *Limnaea* species and, in the cases of *L. palustris* and *L. truncatula*, these have been subjected to statistical analysis.

Most of the shells here described have been collected by me from farms, commons, and roadside ditches scattered over England and Wales. I am indebted to a large number of farmers, veterinary surgeons, and agricultural officers for information, advice, and permission to collect. In

particular, the Ministry of Agriculture's Veterinary Advisory Officers have been most helpful. In addition, I am grateful to Prof. R. T. Leiper for samples of *L. truncatula* from Durham and for active assistance in collecting, to Dr. D. O. Morgan for samples of *L. truncatula* and *L. glabra* from Scotland, to Mr. Basil Goodey for a sample of *L. truncatula* from Kimpton, and to Mr. C. Oldham for a sample of very large *L. truncatula* from Scotland and also for generously giving an expert opinion on various supposed samples of *L. truncatula*.

Nomenclature.

There appears to be no agreement over the nomenclature and systematics of the limnaeid snails. Apart from numerous variants on the spelling of the name *Limnaea*, the genus has been split into several subgenera and by some these are regarded as distinct genera ; thus, Mehl uses the names *Galba truncatula* and *Stagnicola palustris*. The contrary tendency is personified in Brockmeier who regards these as one and the same species.

The problem is one for malacologists, and in this paper I shall follow the nomenclature of Ellis (1926), for reference to whose "British Snails" I am indebted to Mr. G. C. Robson, of the British Museum. The following list of snails referred to in the present paper contains a few of the commoner synonyms :

1. Limnaea truncatula (*Buccinum fossarum, Galba truncatula, Limnophysa minuta, Stagnicola minuta*).
2. Limnaea palustris (*Buccinum palustre, Galba palustris, Stagnicola palustris*).
3. Limnaea pereger (*L. peregra, Radix pereger*).
4. Limnaea stagnalis (*Helix stagnalis*).
5. Limnaea glabra (*Buccinum glabrum*).

A further species, *L. auricularia*, also occurs in the British Isles, "In rivers, lakes and canals" (Ellis), but no specimens have so far come into my hands. A number of varieties are recognised by malacologists, especially in the species *L. pereger* for which Ellis quotes 20 varietal names and three more which may be distinct species. But shell forms may be moulded by environmental influences and, altogether, the difficult art of assigning varietal names is best left to the expert.

Methods.

Living snails were brought or posted back to the Institute in glass tubes lined with white paper which had been wetted, this providing sufficient moisture to keep the snails alive if they were not too numerous. A 3-inch by 1-inch tube will carry up to a dozen small *L. truncatula*, the only danger being that the death and putrefaction of one snail quickly kills the others. Mostly, the snails were used for various experimental purposes and the shells retained after their death. If dead snails are allowed to rot in water for a few days the entire body is readily washed out by a small jet of water. The shells, cleaned and dried, are best kept lying on cotton wool in flat boxes ; if stored loosely, the edge of the shell mouth is liable to get broken.

BIOMETRICAL METHODS.

The following dimensions, shown in Fig. 1, were measured as a routine : total length of shell (symbolized throughout this paper as L), breadth of shell (B), length of the last or " body " whorl (W), and the length (l) and breadth (b) of the shell aperture. From these the following ratios were calculated by slide rule : L/B, L/W, L/l, l/b.

Originally all dimensions were measured by means of sharply pointed dividers with 5-inch legs, transferred to a steel scale engraved in 0·5 mm. With the help of a lens it is possible to estimate to 0·1 mm. but the errors involved in setting the dividers probably amount to $\pm$0·1 mm. Satisfactory as this is with dimensions of the order of 10 mm. it becomes decreasingly so with smaller dimensions. Specimens of *L. truncatula* 6 mm. long have an aperture breadth of about 2 mm. and here the error may amount to $\pm$5%. Accordingly, shells shorter than 5 mm. were not measured.

An improvement was gained by using, for L and B, callipers reading by vernier to 0·1 mm., retaining dividers for the other dimensions. Attempts were made to measure optically with a microscope and eyepiece-micrometer but the low-powered objective (Leitz No. 1a) necessary to include the larger shells had such a depth of focus (the dimensions of the image varying with the depth) as to make the method impracticable. Theoretically, one might measure L with callipers and then determine the other dimensions optically, relative to L ; but in practice it is difficult to hold the shell steady on the microscope stage so that all the required dimensions lie

in one plane parallel to the stage, especially as L and W are not coplanar with 1 and b.

The dimension B is difficult to define unambiguously so as to make it readily measurable, especially with dividers, the points of which are in this case applied tangentially to spherical surfaces. With callipers it was found expedient to use the following method which gave a greater consistency than any other tried. The shell is held so that one calliper blade rests along it (Fig. 1, CC) and is then rotated about its own axis until the outer edge of the peristome also touches the blade ; the second blade is then applied (KK) giving a measurement which, if not at right angles to the long axis, is at least comparable as between different shells.

The dimension W has been taken as lying along the same axis as L and is measured, with the shell aperture facing the observer, from the lower edge of the peristome to the intersection of the first suture with the long axis.

In species of *Limnaea* the shell aperture is not quite coplanar with the long axis of the whole shell, nor is the latter parallel with the plane of the aperture. If the shell is held, apex uppermost and the aperture facing the observer, the outer lip of the peristome is seen to spring abruptly from the body whorl, curving to the observer's right and downwards ; its lower rim is smoothly curved. The inner lip, to his left, is thin and is reflexed over the body whorl ; apically it meets the origin of the outer lip at an angle. The dimension l is taken from this clearly defined angle to the lower rim, and b from the left edge of the inner lip across to the outer lip at the widest part of the aperture and at right angles to l (see Fig. 1).

The outer and lower parts of the peristome are often broken, even in living snails, especially in those from certain localities presumably deficient in lime. In such cases all five dimensions may be affected. In a few samples most shells lacked the extreme apical portion, and the thin conchin layer was missing from this region of the shell : in these the dimension L is alone affected. It was decided to include such shells, since they are found in nature, and to allow their shell deficiencies to contribute to the variation of dimensions and ratios.

Figure 1, the main purpose of which is to illustrate the dimensions used, is a camera lucida drawing of a *L. truncatula* shell from sample No. 15 (see Table XII & Plate).

STATISTICAL METHODS.

These are mainly those described in Fisher's (1936) standard work ; in particular, standard deviations are based on N–1 degrees of freedom. Distributions have had their statistics estimated from grouped data, using arbitrary units. As a number of correlations were to be estimated the data were recorded on cards, one card for each shell, the principal dimensions, ratios or functions thereof being written around the edges of the card for ease in sorting.

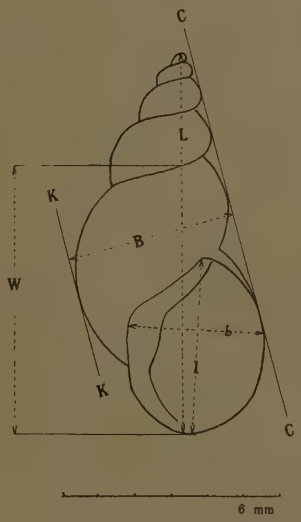


Fig. 1. *L. truncatula* showing dimensions used in this paper.

The ultimate object of this work has been to find ratios which are not merely " constants " descriptive of the species, but of practical use in differentiating one species from another—and particularly *L. truncatula* from *L. palustris*, the only species with which the former is likely to be confused in practice. Such a ratio is, of course, a constant not in the physicist's absolute sense of a precise value to which estimates approximate more and more closely as experimental errors are successively eliminated, but in the biological sense of a population of values in which

variation is an inherent property and of which measures of dispersion (such as the standard deviation) form an essential part. Thus, statistical treatment is necessary. If the mean ratios of two samples are different this fact is of little use until one can estimate the probability of such a difference arising by random variation in two samples from the same population. When this probability is very low it may be fair to assume that the samples were drawn from different populations, i.e., that the difference is significant.

The elementary methods here employed are appropriate to populations whose distributions follow the " Normal " curve. Populations of shell dimensions and ratios might be expected *a priori* to be distributed normally, in which case truly random samples from those populations should also be so distributed. Unfortunately this is not strictly the case with most of the samples dealt with here. Mention has already been made of the fact that it was impracticable to measure shells shorter than 5 mm. : this has the effect in some samples of cutting off the left extremity of the normal curve and of giving a skew distribution with the mode lower than the mean. A hint of skewness in the same sense is seen in the dimensions of 100 *L. palustris*, although all the shells of this sample were measurable ; this may be the effect of unconscious selection of the larger shells when the sample was collected. The skewness is probably of little importance in the estimation and comparison of standard errors of means, especially as most samples are about equally skew, but it may affect the estimates of correlation, which are sensitive to skewness. Correlation statistics must therefore be considered as having comparative rather than absolute value.

Preliminary Data.

Under this heading are included a few small samples, not treated statistically, of various species of *Limnaea* for which the ratios L/B, L/W, L/l, and l/b have been determined. Table I gives in each cell the minimum, mean, and maximum values for each ratio and also for the length L, the extremes being in italic type. At the foot of each column is the number (N) in each sample. No useful purpose would be served by giving the individual data, but these are on file at the Institute of Agricultural Parasitology. The length is important because it is roughly proportional to the age of the snail in any one species, and it is a question for investigation whether or not the ratios are constant for all ages.

The table is useful in showing the large amount of variation to be expected, and also the overlapping of values of a given ratio from one species to another. Absence of overlap is not a *sine qua non* in the practical use of ratios, but its presence implies measuring a number of shells and testing mean ratios for significant differences, the number to be measured depending on the relative size of the difference, the standard deviations, and the level of significance adopted.

TABLE I.—Mean and *Extreme* Values for Ratios in Small Samples of *Limnaea* spp.

Species :	I.		II.		III.	IV.	V.
Sample ...	15.	1.	A.	B.			
	9·9	*5·3*	*10·8*	*5·6*	*10·1*	*16·4*	*4·9*
L ...	12·54	6·59	14·50	7·84	11·35	21·72	7·56
mm. ...	*14·6*	*9·1*	*20·0*	*11·3*	*14·3*	*24·6*	*11·8*
	2·27	*1·85*	*1·79*	*1·75*	*1·56*	*1·71*	*2·22*
L/B ...	2·469	1·928	2·030	1·908	1·686	1·888	2·541
	2·71	*2·09*	*2·28*	*2·10*	*1·84*	*2·16*	*3·01*
	1·43	*1·22*	*1·18*	*1·14*	*1·05*	*1·25*	*1·34*
L/W ...	1·499	1·311	1·265	1·225	1·099	1·284	1·486
	1·57	*1·39*	*1·40*	*1·33*	*1·15*	*1·32*	*1·64*
	1·95	*1·79*	*1·65*	*1·60*	*1·30*	*1·52*	*2·04*
L/l ...	2·135	1·890	1·812	1·741	1·360	1·676	2·278
	2·32	*2·00*	*1·98*	*1·86*	*1·45*	*1·84*	*2·46*
	1·37	*1·31*	*1·46*	*1·34*	*1·40*	*1·49*	*1·44*
l/b ...	1·510	1·438	1·574	1·552	1·494	1·654	1·664
	1·74	*1·58*	*1·70*	*1·73*	*1·59*	*1·84*	*1·78*
N ...	28	20	25	13	15	25	10

Key to species :—
I : *L. truncatula.*
II : *L. palustris.*
III : *L. pereger.*
IV : *L. stagnalis.*
V : *L. glabra.*

For *L. truncatula* and *L. palustris* one sample of large and one of small shells have been included. Here the mean ratios reveal a hint that the ratios may increase with the length of the shell, or in other words that with increasing age the length increases relatively faster than the other dimensions. On the other hand, the apparent correlation of ratio with L might be quite fortuitous or (less improbably) might be associated with the widely different geographical origin of the members of each pair of samples.

The half-tone plate shows, at approximately natural size, shells from a number of samples of *L. truncatula* and from two samples of *L. palustris*. The origin of the *L. truncatula* samples is given in Table XII. Samples No. 15 & 1 are those dealt with in Table I, as are also the two samples of *L. palustris*. Sample No. 15 contains the largest specimens of *L. truncatula* which I have ever seen; they were presented by Mr. C. Oldham whose diagnosis was confirmed by the late Prof. A. E. Boycott. Sample No. 4 is of interest since all the shells were abnormal in lacking the conchin layer around the apex. Sample No. 6 of *L. truncatula* and Sample B of *L. palustris* were separated from a mixture of the two shells occurring in a confined locality in Somerset, but as a rule these two species are not found together in the same habitat. Sample A of *L. palustris* contained 100 shells and is that dealt with in the following section.

The dimensions and ratios here used are not able to bring out the fact that the suture is deeper in *L. truncatula* than in *L. palustris*. This feature can be seen on the Plate, especially if sample No. 15 is compared with sample A.

Data for 100 *L. palustris*.

Early in the progress of this investigation it was realized that more exact treatment of a larger number of shells from one locality was desirable. At that time only a few samples of *L. truncatula* were available, mostly small samples of small shells. *L. palustris* was found in considerable numbers in a circumscribed locality at the north-west corner of Port Meadow, Oxford. The shells were large and firm, lending themselves to more accurate measurement, and the species was one which it was desired to compare with *L. truncatula*.

In the first instance each ratio of the 25 shells in sample A (Table I) was treated as a frequency distribution and the statistics for these are shown in Table II. From this it appears that the ratio L/W is the most satisfactory in that it shows the least scatter (V=3·95%), and the commonly used ratio L/B the least satisfactory: the standard deviation is both absolutely (s=·1328) and relatively (V=6·51%) the highest of the four. There is little to choose between L/l and l/b in this case, but with smaller shells such as those of *L. truncatula* there is a larger proportionate error in measuring b than l, and so the ratio L/l is likely to prove the more useful of the two.

Accordingly the sample of 25 was increased to 100 and the distributions of L, W, l, L/W, and L/l were worked out. Reference has already been made to the fact that the rim of the peristome is often found broken, thus affecting some or all of the dimensions. On this account an attempt was made to eliminate this region of the shell by manipulating some of the dimensions. Thus, two new lengths were added: Y=L−W and X=L−l, and also the new ratio X/Y. The statistics of these distributions are given in Table III.

TABLE II.—Distributions of Ratios for 25 *L. palustris* of mean length 14·50 mm.

Ratio	Mean ± S.E.	s.	V%
L/B	2·030 ± ·0264	·1328	6·51
L/W	1·265 ± ·0100	·0500	3·95
L/l	1·812 ± ·0167	·0835	4·61
l/b	1·574 ± ·0143	·0716	4·55

TABLE III.—Distributions of Dimensions and Ratios for 100 *L. palustris*.

Variate	Mean ± S.E.	s.	V%
L, mm. ...	13·879 ± ·1760	1·738	12·4
W, mm. ...	11·022 ± ·1163	·912	8·3
l, mm. ...	7·765 ± ·0846	·834	10·7
Y, mm. ...	2·857 ± ·0688	·672	23·6
X, mm. ...	6·124 ± ·1012	1·002	16·4
L/W ...	1·257 ± ·0044	·0440	3·58
L/l ...	1·790 ± ·0084	·0829	4·63
X/Y ...	2·182 ± ·0231	·2287	10·48

Of the dimensions, W has the most compact form (V=8·3%) and this must largely determine the low variability of the ratio L/W. The dimensions obtained by subtraction, X and Y, are disappointingly scattered. The standard deviations are satisfactory, that of X less than that of L and of Y less than of W, showing the improvement gained by eliminating the peristome; but the large absolute reduction in the means, due to subtraction, has made the coefficients of variability unduly large. This high degree of scatter is reflected also in the ratio X/Y (V=10·48%).

Compared with the sample of 25 (Table II) the ratios L/W and L/l have lower standard deviations, but the values of V are not reduced proportionately (not at all for L/l) owing to the fact that the means are lower in the larger sample.

The general conclusion so far is that the ratios L/W and L/l are satisfactory in being the least dispersed of those investigated.

COMPARISON WITH THE DATA OF MOZLEY.

Mozley's 1935 paper, which is concerned with the differential proportion of local to total variation as between *L. palustris* and *L. emarginata*, contains data for 1,012 shells of *L. palustris* from 29 localities, the number of shells per locality varying from 1 to 271. The data given are the frequency distributions of four ratios: L/B, L/l, l/b, and B/b, but the statistics for these are not published: " The standard deviation and coefficient of variation have been calculated for most of the series, and will be placed on file at the Smithsonian Institution and the Royal Society of Edinburgh." The interesting conclusion is reached that in

TABLE IV.—Distributions of L/l in *L. palustris*.

Authority	Sample No.	N.	Mean ± S.E.	s.	V%
Mozley	9	50	1·997 ± ·01716	·1204	6·04
,, ...	14	67	1·784 ± ·01136	·0917	5·14
,, ...	20	209	2·057 ± ·00723	·1034	5·04
,, ...	25	271	2·016 ± ·00701	·1143	5·68
Peters	A.	100	1·790 ± ·00841	·0829	4·63

L. palustris the range of variation in any one locality approximates to the range over the whole territory sampled, whereas in *L. emarginata* the variation from one locality to another is a prominent feature; the whole body of data would appear to lend itself to treatment by Fisher's " Analysis of Variance " method.

Apart from length, the dimensions on which Mozley's ratios are based are defined slightly differently from those used in the present paper; but of the four the only one available for comparison is L/l and Mozley's l, defined as the length of the orifice along a line parallel to the axis of the shell, is practically the same as mine. I have selected four of Mozley's larger samples so as to show both similarity and contrast with mine in the mean value of L/l, and have worked out the statistics shown in Table IV.

I am in no way disputing Mozley's thesis that local variation is much more marked in *L. emarginata* than in *L. palustris*, but it is clear that in the absolute sense *L. palustris* shows considerable local variation, and this fact may cast some doubt on the usefulness of such ratios as species constants. They may, on the other hand, prove valuable in defining local races, a question which will be touched upon later in relation to *L. truncatula*.

THE STRAIGHT-LINE RELATIONSHIP.

In putting forward ratios such as the above as species constants—and they are widely so used—certain elementary mathematical assumptions are involved. Taking the generalized equation of a straight-line relationship as :

$$y=ax+b,$$

x and y are the variables, a measures the angle made by the line with the x axis (with suitable scales for x and y, $a=\tan\theta$), and b measures the value of y when $x=0$ (i.e., where the line cuts the y axis).

The ratios are of the simplified and inverted form :

$$x=ay,$$

where x=length, y=some other dimension, and a=the ratio and is the reciprocal of the former a. The assumptions presupposed are (i) that the relationship between x and y is in fact rectilinear and (ii) that the straight line passes through the origin, i.e., that $b=0$. Given the truth of the first, the second is a reasonable sequel in cases like the present, since as length approaches more and more closely to zero other dimensions must do the same. Conversely, if a line based on limited data excluding low values of L appears not to pass through the origin, the consequent *reductio ad absurdum* will discredit the straight-line hypothesis.

It is now proposed to bring evidence from the sample of 100 *L. palustris* showing (i) that the relationship is not rectilinear and (ii) that if it is so regarded a straight line fitted to the data will not pass through the origin. The data used will be L ($=x$), W, and 1 ($=y$).

Variation of Ratios.

If L bears a constant relationship to other shell dimensions then the ratios will be subject only to the random variation inherent in biological data. Reference has already been made above to Table I where there is a strong hint that ratios increase with length but where the hint cannot be

confirmed since the samples come from different localities. We now have samples of 100 ratios (L/W & L/l) both drawn from the same locality, and here, again, the individual data strongly suggest that ratios increase with length. Mozley must have had this in mind when he guardedly states (p. 455): "It has been found that there is little change in these ratios during the latter part of the life of the animal." The shells in the present case were all those of fully adult snails (L=10—20 mm.), and yet the ratios reveal to the eye a tendency to increase with length. This point has been investigated statistically by the method of correlation. Testing the correlation of L with a ratio, there should be no significant correlation if the ratio is constant throughout the growth of the shell. Conversely, if the ratio increases or decreases with increasing length a significant positive or negative correlation should result.

For the present purpose the distributions of L, L/W and L/l are required in order to construct two correlation tables. The statistics of these distributions are included in Table III. Their correlation is estimated by the ratio of the summed product deviations to the geometric mean of the sums of squares of the separate deviations. Thus, if the deviations of two variates are respectively x_1 and y_1, an estimate of the correlation between the variates is given by:

$$r = \frac{S(x_1 y_1)}{\sqrt{S(x_1^2) \cdot S(y_1^2)}},$$

where S indicates summation. Significance is tested by the distribution of a quantity t given by:

$$t = \frac{r}{\sqrt{1-r^2}} \cdot \sqrt{N-2},$$

which has the same distribution as "Student's" t—the ratio of a mean to its standard error calculated on the available degrees of freedom. Fisher's table of t is entered under $n = N-2$. Comparison of values of r from different samples is accomplished by transformation into the quantity z, where:

$$z = \tfrac{1}{2}\{\log_e(1+r) - \log_e(1-r)\},$$

since z is distributed approximately normally whilst for small samples

and high correlations r is not. As r changes from -1 to $+1$, z changes from minus to plus infinity.

The correlation data for L/W and L/l and their respective association with L are given in Table V. This shows a marked and significant positive correlation between length and the respective ratios. Fisher's table of t shows that, for $n=98$ (N=100), t would take by chance a value as high as about 2·6 only once in a hundred trials. The present values of t (7·22 and 5·65) render negligible the probabilities that the estimated

TABLE V.—Correlation of Ratios with Length in *L. palustris*.

Variates	r.	t.	N-2	z.
L & L/W ...	·5895	7·22	98	·6769
L & L/l ...	·4955	5·65	98	·5433

correlations would arise by chance from (in fact) uncorrelated data. This t-test is one application of the " null hypothesis " ; the hypothesis is made that the data are in fact not correlated so that the apparent correlation is due merely to chance. If so, the calculated t should take its tabulated value with the given probabilities. If, at a probability as low as ·01, the calculated t exceeds its tabulated value, it is highly improbable that the null hypothesis is correct ; i.e., the correlation is not fortuitous but real.

Thus, it is clear that both L/W and L/l increase with L so that the shells become relatively longer in shape as they grow in size. This being so, it is no longer tenable that the straight-line law applies : some sort of curve is appropriate.

The Fitted Line.

For the same 100 shells of *L. palustris*, length has been plotted against both W and l in the form of a dot diagram (see Fig. 2). Unbroken straight lines are drawn from the origin satisfying the equation $x=ay$ where, for the relationship of L with W, $a=1·257$ and, for that of L with l, $a=1·790$ (these are the mean ratios of Table III). For both lines it will be noticed that dots representing small shells fall mainly below the lines and those representing large shells mainly above. This poorness of fit is, of course, due to the correlation of length with ratio described above.

Reverting now to the generalized equation of the straight line, $y=ax+b$, it is possible to fit such a straight line to the data by the method of least squares. By this method values for a and b are found such that the sum of squares of horizontal or vertical distances of dots from the line is a minimum. Ordinarily the lines so fitted will differ according as horizontal or vertical distances are selected, the discrepancy between the two lines decreasing as the correlation between the variates increases. The lines are, in fact, regression lines and the a of the equation is the regression coefficient: $a=rs_y/s_x$, where s_y and s_x are the standard deviations of the two distributions and r is the coefficient of the correlation between them.

The existence of two lines rather than one is due to variation and is thus a property inherent in the data. It amounts to whether one chooses, e.g., to express L in terms of W or vice versa. I am indebted to Dr. J. O. Irwin for the information that it is possible to fit an unambiguous straight line by making a minimum the sum of squares of distances of dots from the line, where the distances are measured perpendicularly to the line instead of vertically or horizontally. This line, which runs between the two regression lines, is given by the equation:

$$\tan 2\theta=\frac{2rs_xs_y}{s_x^2-s_y^2},$$

where θ is the angle made by the line with the x axis and the line passes through the means. In cases of high correlation like the present, where the regression lines are separated by only a small angle, no special advantage attaches to the more troublesome use of this third line.

In the case of the regression lines the coordinates of one point on each line are known, viz., the values for mean x $(=\bar{x})$ and mean y $(=\bar{y})$, and from these b can be found by substitution in the straight line equation:

$$b=\bar{y}-a\bar{x}.$$

In order to calculate the value of a in the present case the correlations of L with W and of L with l must be investigated. In the last section the correlations were not obvious and would have been insignificant if the straight-line law had held: the object in investigating them was to see if there was in fact any significant correlation. In the present case the correlation is obvious and the object is to measure its strength so as to calculate a. The data are shown in Table VI and from them the broken lines have been drawn in the dot diagram (Fig. 2). It will be seen that

these lines fit the swarm of dots far more symmetrically than the unbroken lines of $x=ay$. It will also be seen that when $x=0$ (i.e., when the length of the shell is zero), y still takes positive values. These values are smaller in the case of the lines making the larger angle with the x axis but the point is that the ratio lines fall outside the range of the regression lines,

TABLE VI.—Relation between L & W, and L & l in *L. palustris*.

Relationship :			$y=a_1x+b_1$		$x=a_2y+b_2$	
x.	y.	r.	a_1	b_1	a_2	b_2
L.	W	·9469	·6253	2·296 mm.	1·4338	−1·85 mm.
L	l	·8923	·4226	1·85 mm.	1·858	−0·43 mm.

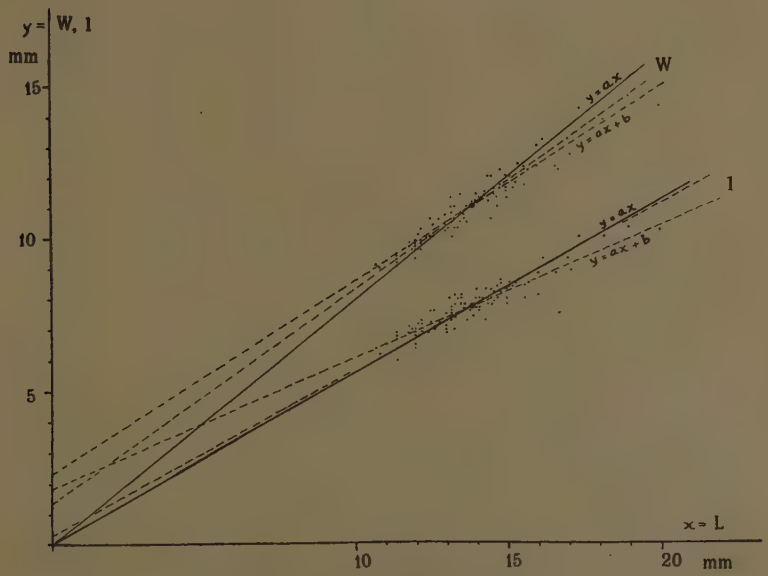


Fig. 2. The relation $y=ax+b$ in *L. palustris*, for L & W, L & l.

particularly in the case of W where the fit is good (i.e., where the regression lines are separated by only a small angle). It should be noted that the values for a_2 and b_2 in Table VI necessarily relate to the opposite axes from those to which a_1 and b_1 relate. With variable material such as the

present the ratio of the means is not necessarily equal to the mean of the ratios, so that the ratio line need not pass through the means as the regression lines must. In this case it so happens that the two values are too close to be distinguishable in the diagram, and as it now appears it was not necessary to determine individual ratios in order to find their mean since the ratio of means would serve; individual ratios were necessary in the previous section, however, where their distribution statistics were required.

It has now been shown that (i) the relationship between length of shell and other dimensions is not in fact rectilinear, and (ii) if it is so regarded a fitted straight line does not pass through the origin, which is absurd.

THE LOGARITHMIC RELATIONSHIP.

The simple ratios L/W, L/l, etc., have been found unsatisfactory since, in addition to random variation, they vary with the size of the shell. In spite of this objection they might be of practical use only where, between two given species, the ranges of variation of a stated ratio showed little or no overlap. Table I reveals considerable overlap in the case of the smaller shells, which are the ones more liable to confusion.

Since the straight-line law is inapplicable it seemed desirable to try the logarithmic law. Wigglesworth (1934), referring to growth in insects, says (p. 87): "As in other animals there is often a change in the proportions of the body as it increases in size; and when this is the case, the disproportion follows the law of disharmony or heterogeny." [Footnote:] "That is, that the logarithm of the dimension of the part is proportional to the logarithm of the dimension of the whole ($y=Kx^a$)." Teissier (1931) has applied this law of the relations of parts to whole in respect of the length, weight, and chemical composition of various insects. Nomura (1927a) has applied it to the length and width of the freshwater bivalve *Sphaerium heterodon* and again (1927b) to those of various molluscs from different localities, including *Limnaea japonica*. Sasaki (1927) has applied it to the length and width of the earshell *Haliotis gigantea*. These examples are from recent literature but the law has a highly respectable past.

The logarithmic relationship:

$$y=Kx^a$$

may be given in logarithmic form, putting $b=\log K$, as follows:

$$\log y=a\cdot\log x+b.$$

This is now the straight-line relationship once more, using log x and log y in place of x and y, and is amenable to the same " least squares " method of fitting.

Teissier calls K the measure of the disharmonic variant and points out that it depends on the units chosen for measuring x and y, whereas a does not. From the previous discussion (p. 191 above) it is clear that log K is the value of log y when log $x = 0$, i.e., when $x=1$. In the logarithmic (straight-line) form, a is again tan θ, measuring the slope of the line. In the original form, if $a=1$ the curve becomes a straight line, $K=\tan\theta$, and y is proportional to x; i.e., there is no disharmony. When a is less than 1, x increases more rapidly than y, and conversely.

TABLE VII.—Nomura's Data for L & B in *L. japonica*. $B=KL^a$.

Locality	N.	a.	K.
1	1261	·99	·537
2	413	1·02	·466

TABLE VIII.—Distributions of log Dimensions in *L. palustris*.

Variate	Mean ± S.E.	s.	V%
log L ...	1·1394 ± ·00534	·05313	4·66
log W ...	1·0407 ± ·00448	·04442	4·26
log l ...	·8873 ± ·00465	·04617	5·21

Nomura uses a different notation but, transliterating, he regards a as a species constant and K as a measure of individual or mean local variation. Equating K_1 from one given locality to unity, the values of K from other localities expressed as ratios to K_1 measure the local variation. His values for length and breadth of *L. japonica* from two localities are given in Table VII. He assumes that a may be taken as unity in this species and adjusts K accordingly: if we follow him we are back at the simple ratios once more with $K=1/a$. It would follow that for this species shape does not vary with size (thus making logarithmic treatment unnecessary) but that it does vary with locality. The latter point will be reconsidered below under the section on *L. truncatula*.

Returning to the 100 *L. palustris*, it is now necessary to investigate the distributions of log L, log W, and log l, and the two correlations : log L with log W and log L with log l. The statistics for the three distributions are given in Table VIII, and the necessary data for fitting straight lines by the method of least squares in Table IX. The individual data and the fitted lines are shown in the form of a dot diagram in Fig. 3. If the three distributions are compared with the first three of Table III it will be seen that a great improvement in variability has resulted from taking logarithms of dimensions. The data of Table IX are comparable with those of Table VI, and reveal a suggestion of closer correlation.

TABLE IX.—Relation between log L & log W, and log L & log l, in *L. palustris*.

Relationship :			$y=a_1x+b_1$		$x=a_2y+b_2$	
x.	y.	r.	a_1	b_1	a_2	b_2
log L	log W	·9596	·8043	·1245	1·1476	−·056
log L	log l	·9072	·7898	−·0124	1·0440	·211

The K of $y=Kx^a$ is the antilogarithm of b in the table and is in no case far removed from unity. This suggests that for practical purposes b might be neglected, taking log $y=a\cdot\log x$, which is equivalent to taking the ratio log y/log x (or its reciprocal) as a constant. The effect of this is seen in Fig. 3 where the ratio log L/log W is shown as a broken line. Comparing Figs. 3 and 2, and assuming that the unknown true relationship between L and W is given by a line lying somewhere between the two regression lines, it will be seen that log L/log W is a much closer approximation than L/W.

In Fig. 2 the angle of the ratio line for L/l was *greater* than that of the regression lines. In Fig. 3 the ratio line for log L/log l is practically coincident with the regression line making the *smaller* angle with the x axis (and so cannot be shown) ; the effect of logarithmic transformation has therefore been greater for L/l than for L/W.

In Figs. 2 and 3 a few of the dots represent two shells with identical values so that there are not 100 dots. It was not easy to indicate this at the scale of Fig. 2, but larger dots have been drawn in Fig. 3 for such double entries, and they will be of the same number and in the same relative positions in Fig. 2.

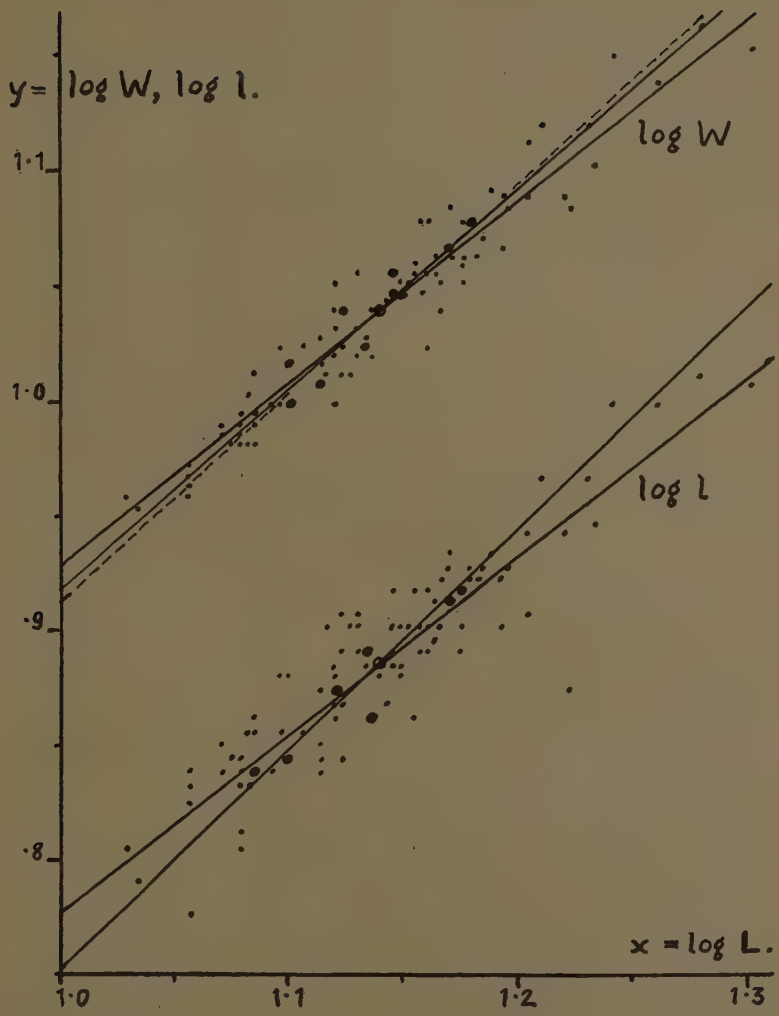


Fig. 3. The relation $y = Kx^a$ in *L. palustris*, for L & W, L & l.

It is unfortunate that tradition has given the ratios the form $x=ay$, where x is length, whereas the straight-line and logarithmic laws give y in terms of x. I have followed tradition, but it must be borne in mind that the straight-line constant a $(=\tan\theta_x)$ is comparable with the reciprocal of a ratio $(=\tan\theta_y)$. To bring them into line with the simple ratios, the logarithmic ratios will therefore be given in the form $\log x/\log y$.

Just as the simple ratios had their distributions (Table III) and their correlation with L (Table V) investigated, so the same must now be done for the logarithmic ratios. The distributions are given in Table X

TABLE X.—Distributions of logarithmic Ratios in *L. palustris*.

Variate	Mean ± S.E.	s.	V%
log L/log W	1·0948 ± ·00144	·0144	1·32
log L/log l	1·2859 ± ·00283	·0283	2·20

TABLE XI.—Correlation of logarithmic Ratios with Length in *L. palustris*.

Variates	*r*.	*t*.	N-2	*z*.
L & log L/log W	·3506	3·706	98	·3639
L & log L/log l	—·0475	·471	98	—

and the correlations with L in Table XI. Comparing the data of Table X with the L/W and L/l data of Table III, it will again be seen that variability has been greatly reduced. Tables V and XI make an interesting comparison. Both the simple ratios show a significant correlation with L, that of L/W being apparently both higher and more significant than that of L/l. Of the logarithmic ratios, log L/log W still shows a highly significant correlation with L, but it is much lower and less significant than that of L with L/W. Log L/log l shows a slight negative correlation with L, but the *t*-test reveals that a value as great would occur merely by chance in about 65% of similar and uncorrelated samples; it is therefore of no significance.

The fact that the ratio log L/log W is not a constant at all ages may be at least partly due to neglecting K. K is the value of y when x is unity, and

there is no necessity for b (=log K) to be zero as in the simple straight-line relationship. At all events the logarithmic ratio is less variable than L/W and is less closely correlated with L ; it is on these counts more satisfactory for practical use. The ratio log L/log l is less variable than L/l and shows no significant correlation with L (this point making it more acceptable as a biological constant), but unfortunately it is considerably more variable than log L/log W.

The preceding pages have dealt mainly with a single sample of *L. palustris* and have been concerned with finding constants of practical use, chosen solely on their inherent merits : ratios chosen for low variability and low correlation with length, and straight-line constants chosen for fitting the data well. The inherent merits of such constants are obviously important, but in the last resort it is their suitability for comparison as between different species which counts. For example, suppose a ratio to have been found for *L. palustris* with minimum variability and no correlation with L : its inherent merits would be of little worth if the corresponding ratio for *L. truncatula* showed no significant difference from it. Accordingly, the methods used above for *L. palustris* will now be applied to *L. truncatula*, and finally comparisons between the two species will be made.

Data for Numerous Samples of *L. truncatula*.

The available samples of *L. truncatula* are mostly small ones. Even where samples originally consisted of 100 or more shells, the majority of shells were too small to be measured with accuracy. However, there is the compensating advantage that the samples are drawn from localities widely scattered over Great Britain, so that the effect of locality, if any, can be investigated. Table XII shows the diverse origin of the 15 samples used, and also the small numbers available.

Although in this paper the *L. truncatula* data follow the others, in fact the work on the two species was concurrent. The writer not then having the results of statistical treatment as a guide, the full dimensions L, B, W, l, and b, and the ratios L/B, L/W, L/l, and l/b were determined for each shell, and only subsequently was it decided that most of these data could be disregarded ; they are all on file, however, at the Institute of Agricultural Parasitology.

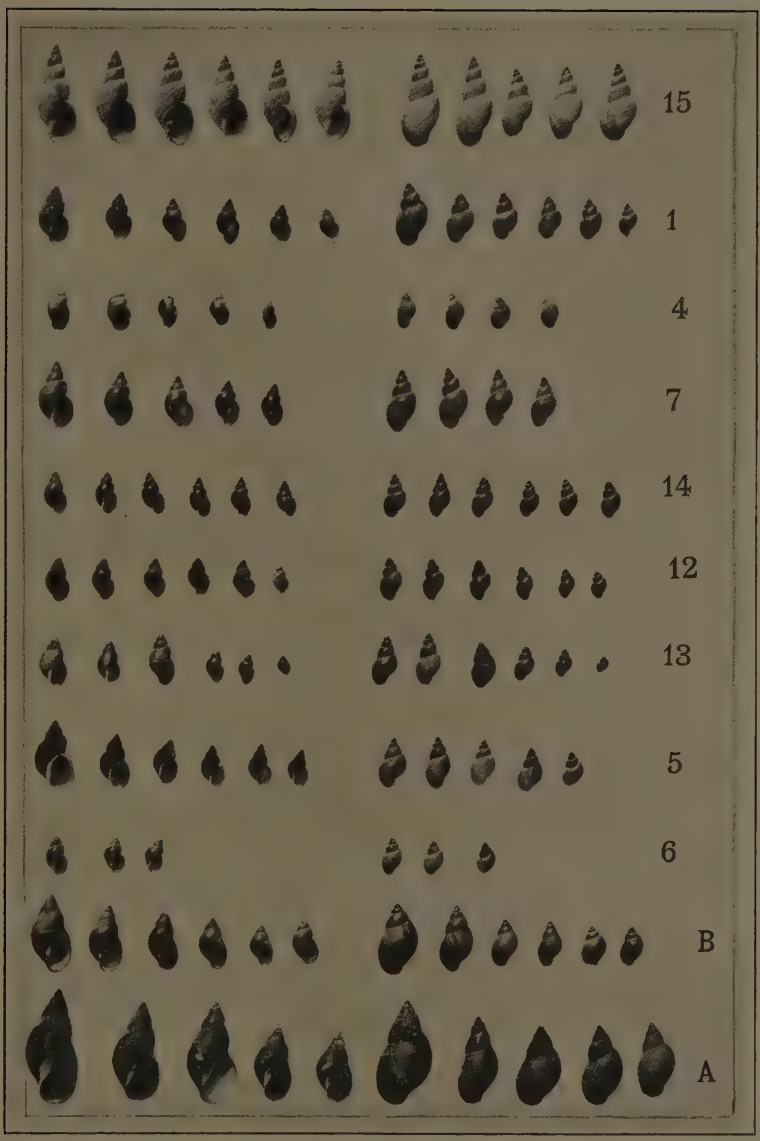
The purpose of the present section is to set out such ratios and straight-line constants for *L. truncatula* as may be comparable with those for

L. palustris. It will therefore not be necessary to give so many data, nor in such disjointed form, as in the previous section. All the required distributions are in Table XIII. They are the two simple ratios L/W and L/l, the corresponding ratios of logarithms, the distribution of L which is required for correlation with these, and the distributions of log L and log W which are required for the *a* and *b* constants of the logarithmic relation between L and W.

TABLE XII.—Origin of *L. truncatula* samples.

Sample No.	Locality	Date of Collection	N.
1	Wytham, Oxon.	27-9-36	20
2	Durham	18-3-37	4
3	Durham	6-5-37	14
4	Kimpton, Herts.	—	9
5	Twyning, Worcs.	19-5-37	16
6	Compton Bishop, Som.	21-5-37	6
7	Wrantage, Som.	21-5-37	9
8	Churcham, Glos.	24-5-37	6
9	Awre, Glos.	24-5-37	3
10	Cowbridge, Glam.	25-5-37	20
11	Raglan, Mon.	28-5-37	3
12	Llanerchymedd, Angl.	5-8-37	12
13	Pentlands, Edin.	7-10-37	20
14	Dunblane, Perth.	12-10-37	15
15	Wick, Caithness	17-7-27	28
			185

So far as variability is concerned, L/W shows a slight advantage over L/l. Log L/log W shows a great advantage over both L/W and log L/log l —the latter being only slightly less variable than L/l. In view of this unexpectedly large variability in log L/log l this ratio has not been further considered.



First nine rows, *Limnaea truncatula* (the numbers refer to the samples of Table XII). Rows A and B, *Limnaea palustris*.

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The correlations of L/W and log L/log W, respectively, with L are shown in Table XIV. L/W has an unusually large and highly significant correlation with L, compared with which the correlation between L and log L/log W is very low and is of slight significance since such a value would occur by chance in about 5% of similar and uncorrelated samples. The same table includes the correlation between log L and log W, required for the *a* and *b* constants. The latter are given in Table XV where for

TABLE XIII.—Distributions of Data for *L. truncatula*.

Variate	Mean ± S.E.	s.	V%	N.
L/W	1·3488 ± ·00597	·08126	6·02	185
L/l	1·9469 ± ·00976	·1322	6·81	184
log L/log W	1·1759 ± ·00192	·02609	2·22	185
log L/log l	1·5121 ± ·00656	·08874	5·86	183
L, mm.	7·585 ± ·177	2·409	31·8	185
log L	·8597 ± ·0091	·1240	14·4	185
log W	·7323 ± ·0077	·1048	14·5	185

comparison the two regression lines are both related to the *x* axis, and data are added for the ratio log L/log W. From this table and from Fig. 4, where logarithms of dimensions are plotted as a dot diagram and the two regression lines are superimposed, it will be seen that the lines are separated by only a very small angle—it is actually 1° 47′.

The dot diagram gives some idea of the goodness of fit of the regression lines. There is a suggestion that a curved line might fit the data better : large numbers of dots fall below the lines at the extremities and above them towards the middle. This may, however, be due solely to the large shells of sample No. 15. All the dots to the right of the broken line belong to this sample and if they were omitted the regression lines would swing round so as to make a larger angle with the *x* axis and the means would drop. Since the immediate object is to investigate the constancy of log L/log W over a range of lengths, sample No. 15 cannot be omitted on grounds of size. It may represent a local race, but the question of local variation has yet to be considered. The ratio line for log L/log W actually

falls between the two regression lines, a fact which amply justifies the use of the ratio in practice—at least with *L. truncatula*.

In 16 cases pairs of shells had identical values and in two cases three shells: these are represented by larger dots of two sizes. Six cases where there were four shells alike are shown as encircled dots, not to be confused with the small square surrounding the intersection of the means.

TABLE XIV.—Correlations in *L. truncatula*.

Variates	*r*.	*t*.	N-2	*z*.
L & L/W	·8341	20·46	183	1·2087
L & log L/log W	·1438	1·97	183	·1452
log L & log W	·9690	53·05	183	2·0751

TABLE XV.—Relation between log L & log W in *L. truncatula*.

Relation	$a_y = \tan \theta_y$	$a_x = \tan \theta_x$	θ_x	b_x	K_x
$\tan \theta_x = r s_y / s_x$	—	·81903	39° 19′	·0281	1·0668
$\tan \theta_y = r s_x / s_y$	1·1463	·87236	41° 6′	—·0178	·9599
$\tan \theta_y = x/y$	1·1759	·8503	40° 22′	—	—

NOTE.—Inferior symbols indicate the axis to which data are referred.

COMPARISON WITH *L. PALUSTRIS*.

The distributions of Table XIII are comparable with those of the corresponding variates in Tables III, VIII, and X. So far as L is concerned, the much greater variability in *L. truncatula* was partly expected since shells varying in length from 5 to 15 mm. were included, whereas the *L. palustris* measured from 10 to 20 mm. Much of the difference may be due to the diverse origin of the *L. truncatula* shells.

The simple ratios, L/W and L/l, also display greater variability in *L. truncatula*, probably reflecting the variability of L with which both are highly correlated. The differences between the means of the four ratios are set out in Table XVI, where the standard error of the difference is compounded from the two by taking the square root of the sum of the sampling variances of the respective means. This table also gives the ranges of the variates and thus reveals the amount of overlap involved.

In spite of the considerable overlap in the ratios L/W and L/l, those for *L. truncatula* being slightly higher, the means give a significant difference as judged by the ratio of the difference to its standard error.

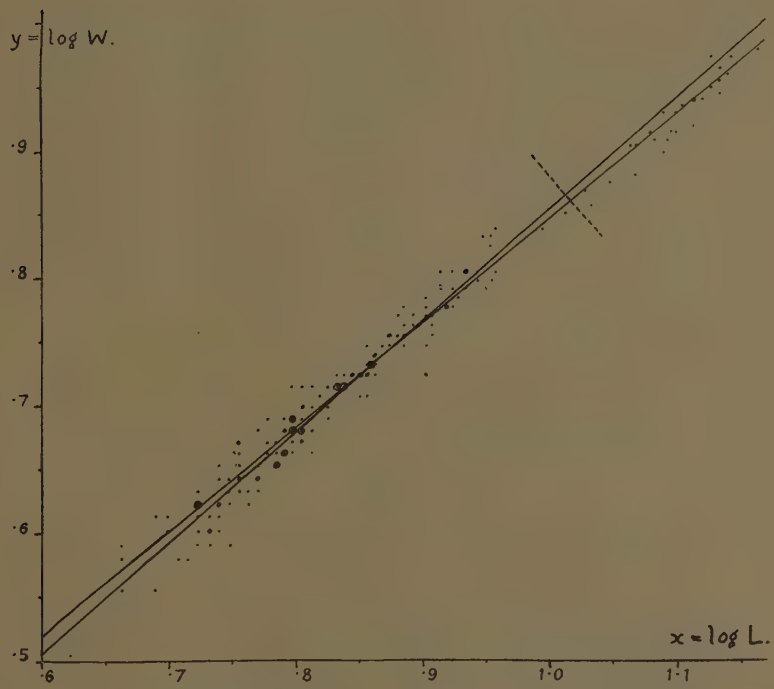

Fig. 4. The relation $y = Kx^a$ in *L. truncatula*, for L & W.

It necessarily follows that the ratios of logarithms are also more variable in *L. truncatula*. These ratios, included in Table XVI, again overlap in their ranges but less markedly than the simple ratios. The differences of means reveal a much higher degree of significance and that for log L/log W although absolutely the smallest of the four, is the most highly significant owing to its low standard error.

Comparing Table XIV with Tables V and XI it appears that L/W is more closely correlated with L in *L. truncatula* than in *L. palustris*, whereas with log L/log W the reverse is the case. What has been said about degree of correlation applies also to significance. A more exact comparison of the correlation coefficients can be made by using the transformed quantity z, the standard error of which can be taken as $1/\sqrt{N-3}$, the standard error of the difference between two values of z then becoming the square root of the sum of the reciprocals of $N-3$. The results shown in Table XVII indicate that the difference is significant in the case of L and L/W, but not in the case of L and log L/log W.

TABLE XVI.—Comparison of Mean Ratios in *L. truncatula* and *L. palustris*.

Variate	Species	Range	Difference of Means	Diff./S.E.
L/W	*L. truncatula* ...	1·18–1·57	·092 ± ·00744	12·3
	L. palustris ...	1·16–1·41		
L/l	*L. truncatula* ...	1·56–2·35	·157 ± ·01281	12·3
	L. palustris ...	1·60–2·04		
log L/log W	*L. truncatula* ...	1·10–1·26	·0811 ± ·00240	33·8
	L. palustris ...	1·06–1·13		
log L/log l	*L. truncatula* ...	1·35–1·82	·2262 ± ·01110	20·4
	L. palustris ...	1·24–1·41		

TABLE XVII.—Comparison of Correlations in *L. truncatula* & *L. palustris*. (For r, see Tables V, XI, & XIV.)

Variates	Species	N-3	1/(N-3)	z.	Diff./S.E.
L & L/W	*L. truncatula* ...	182	·00549	1·2087	
	L. palustris ...	97	·01031	·6769	
		Sum :	·01580	Diff. : ·5318	4·23
L & log L/log W	*L palustris* ...	97	·01031	·3639	
	L. truncatula ...	182	·00549	·1452	
		Sum :	·01580	Diff. : ·2187	1·74

Finally, Tables IX and XV show fairly close agreement in the a and b constants as between the two species. For neither species is it reasonable

to take $a=1$, as was done by Nomura for *L. japonica*. Rather, if simplification is urgent, should K be taken as unity ($b=0$), thus reducing the relationship to a ratio of logarithms of dimensions.

THE EFFECT OF LOCALITY.

Nomura's data suggested that, with *L. japonica*, shape did not vary with size but did vary with locality. With *L. palustris* it was not possible to test this (except that Mozley's data pointed that way), but for *L. truncatula* there are samples from 15 localities, and it was therefore decided to subject the two simple and the two logarithmic ratios to an analysis of variance. If locality has some effect on shape then the total variation met with in the 185 values of a given ratio will be due partly to variation within the individual localities and partly to variation between one locality and another. Now, variance (the square of the standard deviation) has the additive property that the total variance is the arithmetic sum of the component variances ; hence the total variance of any one *L. truncatula* ratio can be analysed into variance between and variance within localities. If there is no locality effect the two should be approximately equal : if the former is significantly higher than the latter then there is correlation between locality and the ratio. Significance is tested by :

$$z=\tfrac{1}{2}\{\log_e v_s-\log_e v_r\},$$

where v_s and v_r are the mean variances between and within localities respectively, obtained by dividing the sum of squares of deviations by the appropriate number of degrees of freedom, n_1 and n_2. Fisher publishes tables giving the values that z would take at probabilities, ·05, ·01, and ·001 for various values of n_1 and n_2. The null hypothesis is that the true value of z in the population is zero ; if so, z will be liable so take the values tabulated with the given probabilities, owing to random variation. If a calculated z is greater than the corresponding tabulated value (at a given probability), it is unlikely that the null hypothesis holds, i.e., the difference between the variances is significant.

Table XVIII gives the data for the analysis of variance of the four ratios in the 15 samples of *L. truncatula*, and the calculated values of z. It will be noticed that in every case the variance between localities is far greater than that within localities. The actual cited values are not comparable as between ratios since they are in arbitary units from grouped

distributions and the width of the grouping is not the same for all. However, if the ratio of (mean variance between) to (mean variance within) is examined it will be found that this ratio is least in the case of log L/log W. In other words, all four ratios are correlated with locality but this correlation is least marked in the case of log L/log W. Fisher's tables of z do not include the precise values of n_1 and n_2 required; but interpolation is unnecessary since, even taking the next available values below (n_1=12, n_2=60), the value of z at probability ·001 is ·5992, and this is much less than any of the four calculated values in the table.

TABLE XVIII.—Analysis of Variance of Ratios in *L. truncatula*.

Variate	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Variance	z.
L/W	Between localities ...	2250·19	14	160·73	1·7725
	Within localities ...	787·16	170	4·63	
	Total ...	3037·35	184	—	
L/l	Between localities ...	1289·06	14	92·09	1·5435
	Within localities ...	710·28	169	4·20	
	Total ...	1999·34	183	—	
log L/log W	Between localities ...	364·21	14	26·01	·8024
	Within localities ...	888·21	170	5·23	
	Total ...	1252·42	184	—	
log L/log l	Between localities ...	923·14	14	65·93	1·4041
	Within localities ...	667·46	168	3·97	
	Total ...	1590·60	182	—	

The lowest value of z is given by log L/log W. The correlation of ratio with locality is therefore highly significant for all four ratios, but is least and has lowest significance in the case of log L/log W. The effect of this on the treatment of the ratios in bulk will be to increase the total variability. The bulking of data is therefore not strictly justifiable, but it does not appear that the use which has been made of it is invalidated: Fig. 4, for instance, based on the 15 samples, gives the impression of greater homogeneity than Fig. 3 based on a single sample.

Discussion.

In differentiating *L. truncatula* from *L. palustris*, simple ratios of shell dimensions and the corresponding logarithmic ratios alike suffer from three disabilities: (i) the ranges of variation overlap as between the two species, (ii) the ratios increase with the size of the shell, and (iii) the ratios vary in different localities. The second and third disabilities are less marked in the case of the logarithmic ratios, the most satisfactory of which is log L/log W; with low inherent variability and correlation with length and locality this ratio combines the most highly significant difference as between the two species.

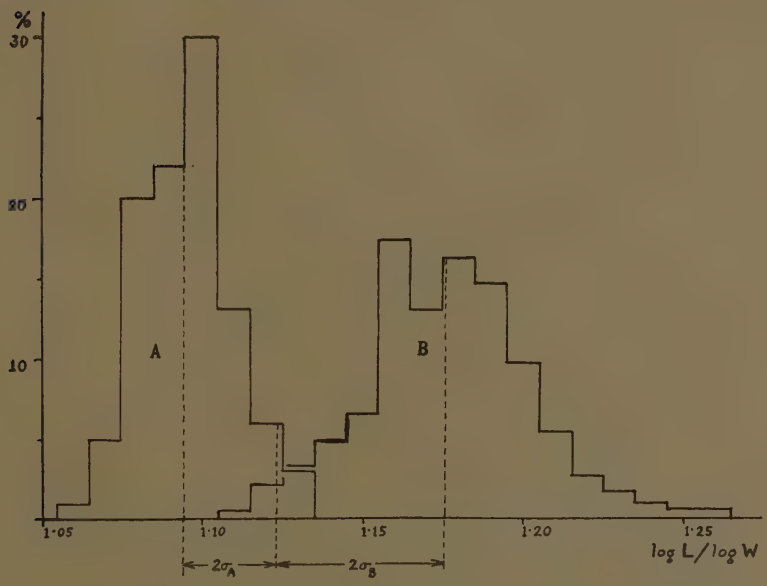

Fig. 5. Frequency distribution of log L/log W in *L. palustris* (A) and *L. truncatula* (B).

The first disability, overlap of ranges, may be considered further with the aid of Fig. 5 which shows in the form of block diagrams the frequency distributions of log L/log W for (A) *L. palustris* and (B) *L. truncatula*. The skewness of A to the right is a reflection of the skewness of the component dimensions to the left, W being slightly more skew than L.

A is relatively compact since the data were drawn from one locality. B is surprisingly symmetrical in view of the fact that it is a conglomeration of 15 samples, but is for that reason less compact. It is probably fair to regard A as a slightly skew sample from a normal population. Since ratios vary with locality there is no special reason for regarding B as normal; but let it be assumed for the moment that both are normal, and that the estimates of the means and standard deviations are the true values of the populations sampled. The probabilities of given values of the normal deviate, in terms of the standard deviation, are known. Thus, the probability of a deviate's exceeding $\pm 2\sigma$ is approximately ·05, and the probability of its exceeding $+2\sigma$ only (or -2σ only) is one half of this. Hence, on the assumptions made, for distribution A about 2·5% of values will lie above the value $\bar{x}_A+2\sigma_A$ (=1·1236) and for B the same proportion will lie below the value $\bar{x}_B-2\sigma_B$ (=1·1237). It is a pure coincidence that in this case $\bar{x}_A+2\sigma_A=\bar{x}_B-2\sigma_B$. This gives a quantitative measure of the degree of overlap, and although the assumptions made are extravagant there would probably be little error in applying them equally to all four ratios for comparative purposes. When this is done it is seen that the degree of overlap is least in the case of log L/log W, where about 2½% of values for one species fall within the range $\pm 2\sigma$ for the other species. This is, of course, an *ad hoc* argument based on present material for comparative purposes; if more samples of *L. palustris* from different localities had been available the overlap between the two species would certainly have been greater, especially as Table I suggests that the young of congeneric species are more alike than the fully grown. The indications remain nevertheless that log L/log W would still show the least overlap of the four ratios.

A species constant which will be liable only to minimal random variation, being independent of length and locality, which will be free from overlap as between different species, and which at the same time will be reasonably simple to determine from small samples, has yet to be discovered. Of the various constants tested the ratio log L/log W comes nearest to meeting these criteria. The a and b constants of the logarithmic relation are doubtless more accurate, but they are more troublesome to calculate, they depend on fairly large samples, and they demand a valid method of computation. If they are to be determined in practice, it is suggested that the method of least squares is appropriate. Nomura (1927a) found his constants by a kind of trial and error method, taking as criterion the

sum of differences of observed and expected x over fixed ranges of y (not, be it noted, squares of differences); Teissier (1931) plotted his data on two-way logarithmic squared paper but the method of fitting the line is not clear.

For the least squares method, the distributions of log x and log y are required and, for smallish samples, are best determined from individual data rather than by grouping. I have found it convenient to construct a table of log x and $(\log x)^2$ where x proceeds in tenths from 1 to 19: this is adequate for all dimensions in millimetres of shells no larger than *L. palustris*. If such distributions are worked out it is not necessary to find also the distribution of log x/log y, unless its standard deviation is wanted, since I have found that (mean log x/ mean log y) is a sufficiently close approximation to mean (log x/log y). If, on the other hand, the a and b constants are not required, log x/log y is a ratio obtainable with little trouble, even from a single shell.

Summary.

1. In differentiating between species of snails various ratios of shell dimensions are frequently used as biological constants descriptive of the species. Some of the ratios commonly used are: length to breadth (L/B), length to length of body-whorl (L/W), length to length of shell aperture (L/l), and length of shell aperture to its breadth (l/b). Mean and extreme values of these ratios are given for small samples of five species of *Limnaea*.

2. In *L. palustris* the least variable of these simple ratios is L/W and the next best is L/l. Statistics are given for the distributions of these two ratios.

3. Data collected by Mozley are used to show that in *L. palustris* the ratio L/l (at least) is subject to considerable local variation, a fact which detracts from its value as a species constant.

4. Statistical treatment of the relations between L and W and L and l in *L. palustris* shows that these relations are not of the straight-line form since each ratio varies with the length of the shell. If they are nevertheless regarded as straight-line relations and if a straight line is fitted by the method of least squares, then W and l take appreciable values when length is zero, which is a *reductio ad absurdum*.

5. By taking logarithms of dimensions, the relation $y=Kx^a$ is reduced to straight-line form thus enabling the constants to be calculated by the method of least squares. The constant K is reasonably close to unity and, regarding it as such, the relation reduces to $\log y=a\cdot\log x$. Unfortunately log L/log W is correlated with length, though the correlation is less than in the case of L/W or L/l. The ratio log L/log l is not significantly correlated with length but is more variable than log L/log W.

6. For 15 samples of *L. truncatula*, a broadly similar condition is found when the data are treated in bulk : L/W is closely correlated with length, while log L/log W is only slightly so correlated. The logarithmic relation between L and W shows that K is fairly close to unity.

7. Comparing *L. palustris* and *L. truncatula* : of all four ratios, log L/log W gives the most significant difference and the least overlap in the ranges of variation.

8. Submitting the 15 samples of *L. truncatula* to an analysis of variance in respect of the four ratios, all four show a significant correlation with locality but this is least marked in the case of log L/log W.

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Habitats of *Limnaea truncatula* in England and Wales during Dry Seasons.

By B. G. Peters, M.Sc., Ph.D.
(*A Principal Research Assistant, Institute of Agricultural Parasitology, St. Albans.*)

Introduction.

Theoretically, the control of a parasite becomes feasible once the details of the life history are known. Since 1882, therefore, when both A. P. Thomas and Leuckart independently described the stages in the life cycle of *Fasciola hepatica*, the essential data for controlling this parasite should have been available. Indeed, there is a widespread opinion that the parasite actually is under control—at least in this country. Chemical substances such as copper sulphate have been used against the intermediary, in some cases successfully, and carbon tetrachloride has proved a valuable anthelmintic against the adult fluke in sheep, largely owing to Montgomerie's pioneer work. All of this is quite satisfactory so far as it goes, and in 1936 J. F. H. Thomas feels able to write: "*Liver Rot* (*Fluke*). Here we can regard the situation with some complacency; as the result [of] the findings of research we know all about the cause, prevention and control of this once so-dreaded disease. All that is needed is that the flockowners themselves should be more zealous in the adoption of preventive and control measures in those areas and during those seasons that the disease is likely to occur." But the older literature clearly demonstrates that fascioliasis has occurred in the past in sudden and severe epizootics lasting one or perhaps two years, and separated by long periods in which the disease is severe only in restricted localities. The notorious epizootic in this country during 1879–80 is a case in point. Following a series of wet years the disease rapidly spread and in one winter destroyed a number of sheep estimated at three millions (A. P. Thomas, 1883A). During this same winter Thomas records that around Oxford the wild rabbit population was almost exterminated by fluke; this is perhaps the cloud's silver lining, but it serves to show the

virulence of the epizootic. Less generalized outbreaks occurred in 1920 and again in 1931, since when heavy losses have been quite sporadic and local.

There can be no doubt that these epizootics are made possible by a sudden and enormous increase in numbers of the intermediary, *Limnaea truncatula*, an increase due in turn to peculiarly favourable climatic conditions. Such an increase is not a mere matter of the snail population's being doubled or trebled. The increase is of catastrophic dimensions, a hint of which can be gleaned from an experience of A. P. Thomas reported in his 1883B paper. He says (p. 7): "During the summer of 1881 I was anxious to try infective experiments with *Limnaeus truncatulus*, but was unfortunately unable to obtain any specimens; the localities near Oxford, where I had formerly found it, were searched in vain. I went out repeatedly in quest of this snail, having on several occasions the skilled assistance of my friend and colleague Mr. W. Hatchett Jackson, but we never found any other trace of this species than the empty shells. It could not be discovered in the localities given for it by Whiteaves in his paper on the 'Mollusca inhabiting the Neighbourhood of Oxford.' My friends at a distance were appealed to but were unable to assist me . . . . In 1882, however, there were floods in July, and the waters of the Isis brought down vast multitudes, probably from its breeding haunts in marshy places up the river. So numerous was it that a single sweep of a small hand-net repeatedly gave me more than 500 examples, and this was in a ditch where last year not a single *L. truncatulus* could be found." It can fairly be estimated, I suggest, that the increase in the snail population in this area was of the order of a thousand-fold at least, allowing for oversights in the careful searches before the floods.

Even periodical doses of carbon tetrachloride will not keep sheep completely free from fluke. The drug may, in a given animal and under optimal conditions, kill all the adult flukes living in the bile ducts at the time, but there will usually be a few young forms migrating which are not killed, and for this very reason repetition of the dose after four to six weeks is recommended. But, in the nature of the case, there can never be complete certainty that all flukes have been killed. In a flukey area, then, sheep are usually carrying a few flukes even in the complete absence of disease symptoms: a fact that is borne out by slaughter house

experience. Cattle also become infected, usually with small numbers, and it is customary not to give these animals prophylactic treatment (especially with carbon tetrachloride), but to treat only on the rare occasions when symptoms are present. The same applies to goats. Apart from these, wild rabbits are of first importance in maintaining a reservoir of infection.

One can readily picture a farm where such conditions obtain. Regular prophylactic dosing of sheep keeps the disease firmly in check, and all appears to be well. But (and this is the point) this satisfactory condition of equilibrium is relative to a given concentration of *L. truncatula* in the infective areas of the farm. That concentration probably fluctuates around a low value for years at a time. But suppose a coincidence of climatic factors favourable to the snail to occur, as it has done in the past, leading to a thousand-fold increase in the concentration of the snail. The equilibrium is at once upset. Massive infections of ruminants and rabbits follow, and an epizootic is the result. In such a case carbon tetrachloride would be, as it is now, a powerful weapon of defence, but in massive infections many deaths occur before the flukes have reached an accessible position in the bile ducts; the disease develops rapidly and the sheep dies, before typical symptoms have had time to appear, as a result of the presence of the young forms in the liver parenchyma. In other words, the damage is done before the drug can be effective.

The object of these introductory remarks is not to deprecate in any way the proper use of carbon tetrachloride, which appears to be one of the best preventives available. What is deprecated is the complacent attitude that fluke is no longer a disease to be reckoned with, an attitude exemplified above in the quotation from J. F. H. Thomas. The view seriously urged here is that fluke is liable to assume disastrous proportions, quite suddenly and over wide areas, whenever climatic conditions are peculiarly favourable to the intermediary. If so, the bionomics of the intermediary and its habitats in dry seasons are appropriate subjects for observation, since (*pace* J. F. H. Thomas) we do not yet "know all about" these things.

In the autumn of 1936 I began an investigation into the factors influencing the distribution of *Limnaea truncatula* and, aided by a grant from the Agricultural Research Council, was able to visit a number of places in England and Wales where fluke is known to have occurred in

the past. The object of this survey was to study natural habitats of the snail under a variety of local conditions, and also to collect specimens for laboratory experimentation. It was very soon evident that a survey in the sense of establishing the detailed distribution of *L. truncatula* throughout England and Wales was quite impossible. Such a survey would occupy a great number of years ; there is good reason for believing that the distribution varies considerably in its details even from year to year ; and even if a detailed survey could be accomplished in one year, it is not clear what particular use could be made of such data. On the other hand, a study of a restricted number of widely scattered habitats might help towards a precise knowledge of the optimal conditions of life for the snail, and this in turn might well suggest new control measures.

As it happens, the period since October 1936 has been specially unfavourable to the snail. In many cases it has been impossible to find a single specimen in a notoriously flukey area. Even the most thickly populated habitats could yield only a dozen snails to the square yard, compared with Thomas's 500 in one sweep of a net. This state of affairs, however satisfactory to sheep farmers, has hindered progress. But, at the same time, the unfavourable weather can be considered as having driven the snail back to its ultimate strongholds, so that the few places where they have been found in any numbers indicate the sources from which they will multiply and spread in wet seasons—the proper objectives in any campaign of control.

The present paper contains an account of the methods used in the survey and a detailed description of some of the more interesting habitats found. In a few cases habitats are included where no snails were found but where circumstantial evidence points to their having occurred in recent years. It has been difficult as yet to arrive at conclusions of much value, and the paper has rather the nature of an interim report.

In discussing farms the name and address of the farmer have been withheld ; farmers have been most helpful and patient, supplying detailed information and conducting me round their farms, but they do not desire to broadcast the fact that their land is potentially dangerous to sheep and cattle. This explains a certain vagueness in describing the localities discussed. For the same reason, farmers can here be thanked for their invaluable assistance only collectively and anonymously. Apart

from farmers, I am indebted to many others for advice and assistance far beyond the mere demands of courtesy ; I shall hope to refer to most of them individually in the text.

Methods.

CONTACTS.

A possible approach to the problem, and one appropriate to a detailed survey, would have been to take a number of widely scattered areas at random and to seek the snail in each area. Since the object was rather to study a variety of known habitats, the approach was in this case by way of definitely flukey areas, since a recent history of fluke probably implies the presence of the snail. The first step was a request for information sent to all the Veterinary Advisory Officers of the Ministry ; I am indebted to them all for helpful replies which in many cases included addresses of flukey farms and of practising veterinary surgeons who would be likely to have local knowledge. Judging solely from these replies it appeared that fluke was most widespread in the three provinces : North Wales, South Wales, and the Western province comprising the counties of Worcester, Hereford, Gloucester, Wiltshire and Somerset. Up to date more farms have been visited in these three provinces than in all the others together.

At the time when this survey began, Mr. R. S. Roberts, F.R.C.V.S., had been investigating braxy-like diseases of sheep under the auspices of the Agricultural Research Council, and had extensively toured the sheep-rearing areas of Great Britain. Although not specially interested in fluke he might possibly have notes on its presence in areas where it was of outstanding importance. In response to a request he very kindly went through his records with me and gave me a large amount of valuable information for which I am greatly indebted to him. Amongst other things, this led to my getting into touch with a number of farmers in the area between Torquay and Plymouth.

Contact with the farmer having been established, the next step was to obtain from him information on sheep management, which varies very considerably from one locality to the next, on movement of animals by purchase and sale, and on any recent history of fluke. In some cases the farmer had a shrewd idea of the source of infection on his farm, and in a very few cases he knew *L. truncatula* at sight and could go straight to its breeding places.

SAMPLING.

Snails were collected and brought or sent back to the Institute of Agricultural Parasitology for experimental use. In some cases samples of water and mud, and of associated organisms, were also collected. At the same time certain routine physical and chemical observations were made. Thus, a note was made of cases where the snails were found out of water, and in all cases the prevailing weather was described; there is probably a connection between the two. The temperature of the water was usually taken and, if the snails were out of water, dry- and wet-bulb hygrometer readings were also taken.

The hydrogen ion concentration of waters containing the snail was determined with the Lovibond Comparator in which a *p*H indicator is added to the sample and the resulting colour is matched against standard Tintometer glasses embedded in a Bakelite disc. This method has proved very useful as a field technique and the apparatus is less cumbersome than that making use of sealed glass tubes of liquid standards; it is just possible to manipulate the apparatus with two hands (standing, e.g., in mid-stream) without the need of setting down bottles and other spare parts on some precarious rock. The following colour discs and indicators were carried about: Chlorophenol Red (*p*H 4·8–6·4), Bromo-thymol Blue (*p*H 6·0–7·6), and Cresol Red (*p*H 7·2–8·8); but I have reached the conclusion that the B.D.H. "Universal" indicator (*p*H 4–11), for which also a disc is available, would be sufficiently accurate for the purpose.

Originally an attempt was made to get some measure of the oxygen requirement of water samples by the use of acidified $KMnO_4$ solution, the measure being the time taken for the sample to fade until it matched a standard made up at half the concentration of $KMnO_4$. There is no clear end-point in such a method and the experimental error is therefore large. Moreover the necessary apparatus added to the luggage disproportionately to the value of the test, which has therefore not been made latterly.

In addition to the thermometer, whirling hygrometer, and Comparator with spare discs and indicators, a number of labelled tubes must be carried, and it has been found desirable to add: a collecting stick with attachable nets, tube holder, and weed-hook; a small trowel and a coarse sieve for dealing with mud samples; a waterproof sheet (so that one can lie prone on a swamp in relative comfort); pairs of forceps, notebook,

hand-lens, and a camera with its necessary paraphernalia. Now, it is simple enough to transport these impedimenta from farm to farm by car, but it may be necessary to walk a considerable distance at any one farm, and experience has taught that time is lost by not carrying *everything* along with one. The ordinary attaché case has the wrong shape for the purpose; it is too deep and its contents are soon in a chaotic state. Accordingly, I have had made an attaché case measuring 12 inches by 24 inches by only 3 inches deep. When this is opened everything lies patent; it is just large enough to contain all the apparatus, and being made of fibre is not intolerably heavy.

RECORDING.

The requirement here was a method of recording the place and time appropriate to each sample, along with meteorological and other

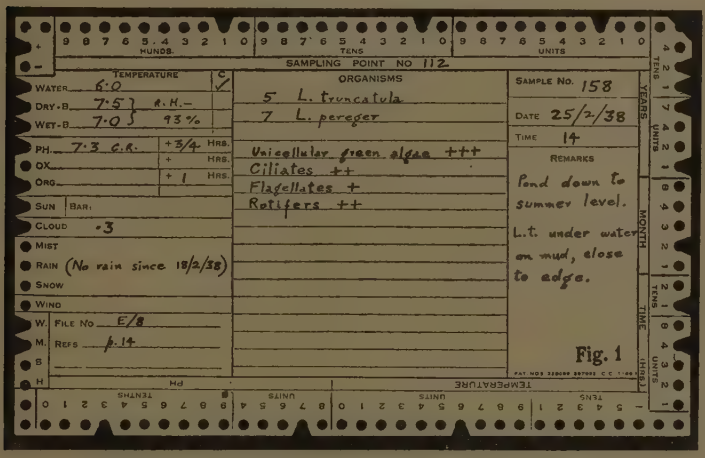

Fig. 1.—Specimen of "Paramount" card duly filled in and punched; one such card is used for each sample.

observations, in such a way that rapid sorting and counting under various classifications was possible. For this purpose 5 inch by 8 inch "Paramount" punched cards were used, prepared by the Copeland-Chatterson Co., Ltd. A specimen card, duly filled in and punched, is illustrated in Fig. 1.

The three-figure sampling point number is written towards the top and is punched out along the upper margin. A numbering scheme

has been devised whereby England and Wales is divided into nine large areas specified by the figure in the hundreds group. These areas are not political but follow (mainly) the larger drainage basins; they are far from equal in size since smaller areas were chosen where fluke was thought to abound, so as to give more detailed subdivision. Each area is subdivided into nine districts specified by the tens group, and each district into nine sampling points specified by the units group. Subdivision again follows river basins where possible so that boundaries usually coincide with water-sheds. "Points" are by no means Euclidean in the sense of having no area and, in the larger districts, a point may cover several square miles; however, a given number (such as 772) does stand for one definite bit of ditch, or a pond, or a swampy patch, and any other habitats in the same "point" are distinguished by alphabetical suffixes. To give an example: 700 stands for the Severn basin in general; 770 is the north-west bank and its tributaries up to Tewkesbury, in general; 772 is the basin of a small river opening into the Severn at Awre. But, as used on a sample, 772 is a ditch where *L. truncatula* was found on a certain farm in that basin, and other collecting places in the same basin are distinguished as 772a, 772b, etc.

It has been helpful to the memory to keep rough sketch-plans of farms visited, showing where samples were taken and where *L. truncatula* was or was not found. These are drawn on the back of Paramount cards of a different tint, used as guide cards. There will be one guide card for each locality and as many sample cards as there are samples. All cards bear the sampling point number in order of which the cards are filed. The punching not only facilitates filing but provides a quick method of sorting under various classifications. Thus, if cards are for some purpose arranged in order of date, and if the Severn basin cards are wanted, it is only necessary to run a knitting needle through hole No. 7 in the hundreds group, and lift the cards therewith, when the 700 cards will all fall away, since they are punched out at that hole.

To the right of the cards are spaces for sample number, date, and time. The sample number is simply a running number of special value when samples have been taken on different occasions from the same point: as it is roughly proportional to the date, it is not punched. The year and month are punched by a more compact system than the point number, and so is time of day by the 24-hour clock, but not the day of the month

since one would not normally want to *sort* under this category; there might well be some characteristic common to samples taken in any January, or taken early in the day (low temperature, for instance) but scarcely one common to any 14th January.

Top left are the signs + and — to signify presence or absence of *L. truncatula*. Below are spaces for water temperature and hygrometer readings; at temperatures above zero Centigrade the water temperature is fully punched along the right bottom margin, while at lower temperatures the dry-bulb reading is punched; confusion in sorting is obviated by the minus sign on the extreme right. Below the temperatures are spaces for *p*H and oxygen requirement, and a space to show whether or not organisms associated with the snail have been listed in the central part of the card; if these three determinations were not made on the spot, the time lapse is noted. When *p*H readings are taken they are punched to the left of the lower margin.

There follow six spaces for meteorological data other than temperature, usually qualitative only, and finally four holes to indicate whether snails were under water, and also the nature of the substrate. These are lettered W (water), M (mud), S (stones), H (herbage), and any of the three substrates can occur with or without W.

The scheme of filing is completed by a register of sampling numbers which also functions as a diary of collecting expeditions, and a numerical register of sampling points accompanied by a large map.

Individual Habitats.

In this section will be given a detailed account of some individual habitats, or supposed habitats, of *L. truncatula*, selected for their interest. In most cases the unit for description will be a single farm and the units will be broadly grouped in geographical areas which for the most part are the provinces recognized by the Veterinary Advisory Officers; this is more convenient to the present purpose than following river basins. Where maps are shown, they are to be taken as rough sketches only, bringing out essential features, but probably unrecognizable by the farmers concerned. On all maps the encircled sign + indicates the places where *L. truncatula* was found.

A. South Wales.

I am deeply indebted to the Veterinary Advisory Officer of this province, Capt. N. Bisset, M.R.C.V.S., for his generous assistance to me during a

tour in May, 1937. Apart from putting me into touch with practising veterinary surgeons and farmers, at a distance from Cardiff, he gave me for a whole day the services of his personal assistant, Mr. Gubb, to whom I am also grateful; knowing the district around Cardiff well, he most efficiently discharged the offices of escort and guide in a rapid survey of the flukey parts of the district.

There are many patches of known flukey land in South Wales, and I was enabled to see some of the worst of them. In spite of this selection it was very difficult in most places to find *L. truncatula* at all—a fact which Capt. Bisset was able to confirm from his own experience—though some years previously the snail had been very abundant.

Case 1.

From Cardiff a narrow road runs along the coast towards Newport, quite distinct from the main road which it leaves at Rumney. The country here, known as the marsh, is an Alluvial flat and is only a few feet above sea level. It is drained by deep, wide ditches known locally as "reens" which traverse meadows where sheep and cattle are grazed, and of which one runs alongside of the road for some distance. This particular reen carried a heavy growth of mixed water weeds upon which aquatic snails were numerous. Extremely common in places were *Bithynia tentaculata* and *Limnaea pereger*, and there were many planorbids. In other places, near farms where domestic ducks were kept, snails were scanty. Prolonged search revealed only two specimens of *L. truncatula* and it was obvious that this was not a normal habitat for that species. They are seldom found in streams more than a few inches in depth. At one point a reen at right angles to the road ran towards the sea through a meadow where sheep were grazing. There were very few snails of any kind here, and no *L. truncatula*, possibly owing to the infiltration of sea water. It seemed likely that in wet seasons when the small ditches tributary to the reens presumably contain water, *L. truncatula* might find in these a satisfactory habitat. They would probably be brought down via the reens from the higher ground up towards the main road.

One farm on this higher ground was visited. Fluke had been severe there some years previously, but not a single *L. truncatula* was found on this occasion. Ditches were well cleared and there was a good fall down towards the marsh.

Case 2.

This was a farm not far from Cowbridge on rising ground close to the 200 ft. contour line. The feature of special interest was a large expanse of rough pasture rising fairly steeply, from a small river bordered by marshy patches in the south-west, to a wood in the north-east. The field is shown diagrammatically in Fig. 2. The soil is a grayish clay which may belong to the underlying Lower Lias, although glacial drifts occur in the region. The vegetation consisted of coarse grasses,

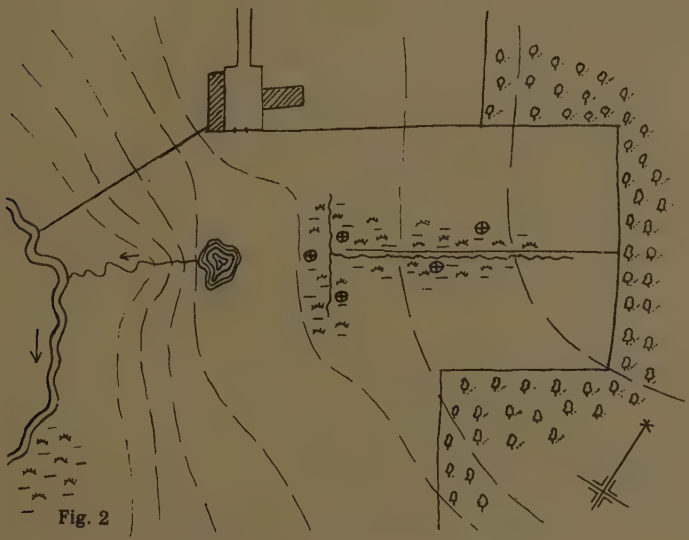

Fig. 2.—Diagrammatic plan to illustrate Case 2.

sedges and rushes, and large patches of the wild bog-iris. Near the farm buildings was a duck pond overflowing by way of a small stream down over the slope to the river, which had a fairly rapid flow. The pond itself, the streamlet, and the river were all negative for *L. truncatula*, as was also a marshy patch adjoining the river. On the higher ground up towards the wood there were boggy patches, drained to a slight extent by shallow ditches, and here the snail was found in fair abundance, up to a half dozen to the square foot. Some of the snails were immersed in

water that was nowhere more than two inches deep ; others were out of the water on damp mud close at hand. Although no sphagnum was seen, the general impression was of decidedly acid land, and I was therefore surprised to find that the reaction of the water was *p*H : 7·3. The farmer knew that this field was dangerous and he kept sheep away from it.

Twenty of the shells from this locality were measured and had an average length of 7·00 mm., varying from 5·6 to 9·0 mm. They were well formed and of a firm consistency, but slightly more slender than is usual ; the ratio of length to breadth was 2·03, whereas the average for the species is, in my experience, rather less than 2.

Case 3.

Capt. Bisset kindly gave me an introduction to Mr. C. Watson, M.R.C.V.S., practising in Haverfordwest, who was good enough to receive me at a very late hour, to discuss local fluke problems, and to give me addresses of likely farms in Pembrokeshire. The winter of 1936–37 had been noticeably bad in the county. The prevalent custom of farmers' not seeking professional advice for fluke, which they usually treat themselves, was broken by several, one of whom had lost a valuable ram in addition to several ewes. Mr. Watson had seen the ram but had been called in only when it was too late to save it : it was heavily infested with fluke.

I visited this farm, which stands on the side of a hill sloping south down to a tributary of the Western Cheddau (Fig. 3), the underlying geological measures here being Ordovician. A small trickle from a spring near the farm buildings found its way by a series of small boggy terraces down the steep slope to the river, along the banks of which were several large boggy patches. The situation seemed well suited to *L. truncatula* for, while I have not found it favour stagnant acid swamps, the boggy patches on the hillside washed by a gentle trickle from the spring seemed ideal. I worked down from the farmstead to the river, following the course of the streamlet, but not a snail of any kind could I find. It appeared that the same course had been even more thoroughly worked over before me by the farm ducks. The stagnant swamps along by the river were similarly negative. Yet fluke had been rampant on this farm during the previous winter and it appeared to the farmer, remembering where his sheep had grazed the previous summer, that the disease could have been picked up only on the low meadows by the river. The ducks

must have been uncommonly active in the interval ! And, since there are always ducks to deal with the slopes, the snails could only arrive by way of the river. Here there was a swift flow, but plentiful water weeds were growing in the river bed, spread out like a net to catch any snails that might be swept down from higher reaches. And the weeds were swarming with many thousands of very small snails of the *Limnaea* shape. The reaction of the water was pH : 7·2. The snails measured from 1 to 2 mm.

Fig. 3.—Diagrammatic plan to illustrate Case 3.

in length and species of *Limnaea* cannot be distinguished at that size ; so a considerable sample was dispatched, without further examination, to the Institute for culturing. When I eventually returned to the Institute the snails were all dead.

If only I had paused to use a hand-lens when these snails were collected the awkward moment might have been avoided when the expert eye of Mr. C. Oldham recognized the shells as those of young stages of *Hydrobia jenkinsi*. I have found this operculate snail many times, before and

since, and although the adult (about 5 mm. long) somewhat resembles a young *L. truncatula* in shape, the peristome is more nearly circular in outline, its outer lip not springing abruptly from the body-whorl as it does in *Limnaea*, and the whorls are more numerous. Moreover, there is an operculum which, however, may be withdrawn well within the peristome and so be not easily detected, and there are filiform tentacles unlike the squat, triangular tentacles of *Limnaea*. It is easy to see now that shells under 2 mm. in length are readily identifiable in this species. *Hydrobia jenkinsi* is of particular interest, not only as the only parthenogenetic species of mollusc known, but also as having changed its habitat since the end of the nineteenth century when it was exclusively a brackish water species.

Case 4.

Another farm, situated between Haverfordwest and St. Clears on Devonian measures, resembled the previous one in the recent history of fluke and the apparently complete absence of *L. truncatula*. For an introduction to this farmer and others I am indebted to the kindness of Mr. G. Rowe, M.P.S., who is in business in Narberth and takes a close interest in fluke (for which he dispenses a remedy of his own). Again, for the introduction to Mr. Rowe I am indebted to Mr. Watson of Haverfordwest. The topography of this farm was also somewhat similar to that of the last, the farm buildings at the top of a slope running down steeply to the banks of a small river, a tributary this time of the Eastern Cheddau. Here again there was a spring near the buildings and a swampy trickle down over the slope, with more boggy patches in the bottom. The river ran swiftly, was free from weeds, and at this season was shallow enough for wading. During the previous season fluke had been severe in home-bred cattle. A duck pond was completely negative for snails, as is the usual case with duck ponds. Several hours were spent on this farm in a careful search which yielded, in a swamp near the river, a single specimen of *Bithynia tentaculata*, and no other mollusc. Not only were there ducks on the farm but, along by the river, the far bank of which was wooded, moorhens were common. The reaction of the river water was *p*H : 7·0.

It is difficult to account for the absence of *L. truncatula* on flukey farms like this and the last, where the question of drought does not arise, except on the assumption that ducks, and perhaps moorhens also, are

able to keep the snail population down during normal seasons, and that a sudden large increase in the snail population is too much for the birds. These are but two instances of what I have repeatedly found : that snails of all species are very scarce wherever ducks, or moorhens, or both, abound.

Case 5.

In Monmouthshire there is a large number of villages bearing the name Llanvihangel, which may be loosely translated St. Michael, followed by some distinguishing suffix : they seem to take the place of the many

Fig. 4.—Diagrammatic plan to illustrate Case 5.

" Llanfairs " further north. Capt. Bisset had given me the address of a farm near one of these villages where fluke had occurred several years ago. The farm had recently changed hands and the new occupant had seen no obvious cases of fluke among his animals. I did not keep him talking, as sheep-shearing was in progress.

The farm (Fig. 4) was bisected by a railway embankment. Such a feature might be thought to act as an efficient barrier to *L. truncatula*, but I have found that there are numerous streams piped under these

embankments in most parts of the country. On the farmstead side a small stream ran past the buildings, down a gentle slope to the railway under which it was carried by a culvert ; on the other side the stream quickly passed out of the land belonging to this farm. Nearby, on high ground, was a swampy patch which appeared to be a silted-up pond. The swamp was negative for *L. truncatula*, but two small specimens were eventually found in the stream, half way down the slope (pH : 7·3), and this in spite of a flock of ducks which were working systematically along its course. The snails were hidden from direct view by vegetation.

On the far side of the railway on high ground were a large pond and a boggy patch in the same field ; both were negative for *L. truncatula*. Down over a steep slope was a small pond under an oak, fed by a thin trickle from a spring not far away. Spring and pond did not deserve a visit since they regularly dry out every summer. Moreover, on this unpleasantly hot day the Military authorities had staged Infantry field manoeuvres in the area and I was subjected to heavy fire from both sides (apparently with blank cartridges). Leaving behind all gear other than a corked tube, I made the descent to the pond, only to find considerable numbers of *L. truncatula* in it. There were no ducks kept on this side and apart from the solitary oak, it was open meadowland uncongenial to moorhens, but it was nevertheless surprising to find *L. truncatula* in fair numbers in a habitat that dries out every summer. The snails either had moved up in autumn from further down the valley where there was permanent water, or were able to retire into some relatively damp spot and aestivate during the drought. Elsewhere (Case 11) there is evidence for the latter alternative. This and all the other farms I visited in Monmouthshire were on the Devonian measures.

Case 6.

Through the good offices of Capt. Bisset I was put into touch with Capt. W. A. Williams, M.R.C.V.S., practising in Abergavenny, to whom I am grateful for details of two fluke-infested farms in Monmouthshire. In one of these, near Raglan, *L. truncatula* was found in fair numbers, but in a quite typical habitat (a quietly moving shallow ditch) that does not present points of special interest.

The second farm was situated in steep hilly country, at an altitude of about 400 ft., close to the Hereford border. Fluke was regarded as

a serious matter by the farmer, whose sheep were regularly dosed with carbon tetrachloride. Two streams arose from springs on the high land, finally uniting, and falling down the steep slopes in a series of cascades and waterfalls. A curious feature was the presence of boggy patches high up on the slopes. These patches, known locally as "galls," are overgrown with rushes and sedges and are perennially wet, yet no stream flows from them. It is as if a spring emerged and immediately soaked back into the ground again. Some of the galls were in open grass-land and others in wooded spinneys. In the neighbourhood of a gall was to be found a grassy sedge, locally called "Carnation grass" (possibly *Carex panicea*), which the farmer regarded as diagnostic of flukey land.

It was a disappointment to find no *L. truncatula* in such likely spots as the galls, and in fact only a single specimen was found on the farm, high up in the course of one of the streams in a shallow pool. The whole course of the stream was searched including the swampy pool where it rose, but no others were found. It is difficult to imagine *L. truncatula* making its way upstream against such odds as cascades and waterfalls, and seems more likely that this one specimen had been transported on the feet of a water bird.

The great problem (as in so many cases) was to account for the relative absence of the snail. It is true that there was no recent history of fluke. The disease had been prevalent in the past and carbon tetrachloride was used regularly as a prophylactic measure, but it is possible that *L. truncatula* had been scarce or absent from this land for some years.

B. NORTH WALES.

During the August of 1937 I was enabled to visit North Wales. I had for some time been in touch with Dr. R. F. Montgomerie, F.R.C.V.S., then Veterinary Advisory Officer at Bangor, but it was not possible to find mutually satisfactory times for the visit until after he had resigned to take up his new appointment. To his successor at Bangor, Mr. W. T. Rowlands, M.R.C.V.S., D.V.S.M., I owe a special debt of gratitude. Although I arrived rather soon after the change of appointment, Mr. Rowlands sacrificed several days to escorting me around, his detailed knowledge both of veterinary matters throughout the province and of the Welsh language proving invaluable. He also gave me a useful introduction to the Principal of the Madryn Farm School at Bodfean.

Such tours have to be arranged in advance, and it was in some ways regrettable that North Wales was complaining of six weeks of drought conditions at the time of the visit. Aquatic snails were hard to come by ; so much so that I must have begun to doubt my ability to find them had it not been for the reassuring concurrence of Mr. Rowlands.

Case 7.

This was a farm near Llanerchymédd in Anglesey, lying on Ordovician measures. The farmer not only had a good understanding of fluke but was able to recognise *L. truncatula* at sight. The farm was mainly on undulating land standing at an altitude of about 200 ft., but falling away rapidly towards the north to marshy flats (see Fig. 5). The interesting feature of the farm was the fact, for which the farmer vouchsafed, that his flukey fields were on the high land and not down in the flats.

The worst of the flukey fields, at the highest part of the farm, was at this time quite dry, though it lies rather wet in winter. Along the lower edge of the gently sloping field was a drainage ditch : this contained no flowing water but here and there were small residual puddles. Within two minutes the first *L. truncatula* was found in one of these puddles, and several more rapidly followed—contrary to the farmer's expectation for such a dry season. The reaction of one of the puddles was *p*H : 6·9. Many of the snails were found on wet mud at the edges of the puddles. Notwithstanding the absence of rain, the sky was completely overcast, so that drying of the mud would not be as severe as in bright sunlight. The shells were firm and well-formed, the average length of 12 shells selected for measuring being 5·83 mm. (5·1 to 6·2 mm.), and the average ratio of length to breadth 1·97. In addition there were several shells too small for detailed measurement. They were obviously snails of the same year, but there was no trace of their fully grown parents.

In the ditch at the upper side of this field, and in ditches of another field on high ground, the farmer had found *L. truncatula* plentifully in the previous November ; these places are marked with an asterisk in Fig. 5. But on this occasion those ditches were dry and no trace of the snail could be found by any of us. The curious point is that no empty shells were found, after a lapse of less than nine months at most. It seems at least possible that wild birds eat such empty shells as a source of grit for the gizzard. Whatever the reason, it probably also applies to

the South Wales farms where no shells were found, the feature of this farm being the definite knowledge of the snail's presence within the year.

The lower fields towards the flats were of rough grass with many thistles. Water from a spring trickled down these fields into the flats themselves where it emptied into a large ditch of standing water (pH : 6·5), bordered by marshy land carrying rushes and sedges. No snails were found in the spring water, but young stages of *L. pereger* and several species of *Planorbis* were scantily represented in the ditch. *L. truncatula* was not found. The

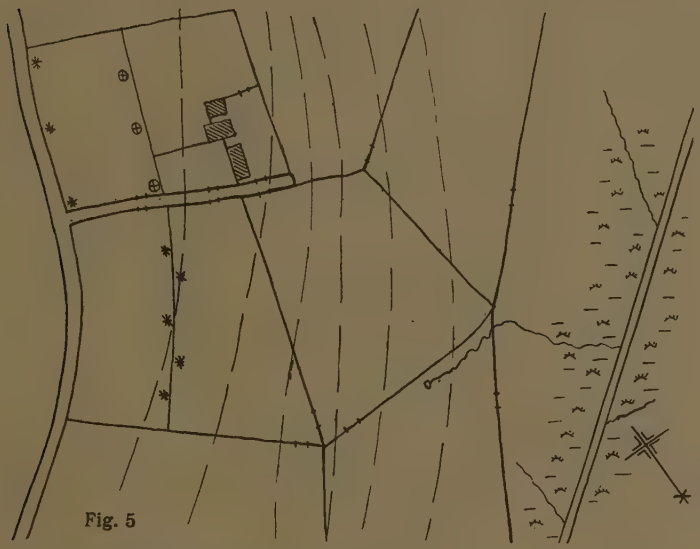

Fig. 5.—Diagrammatic plan to illustrate Case 7; the sign * shows where *L. truncatula* had been found in Nov., 1936.

farmer thought the water in the flats might be acid, but it was only slightly more so than that in the high field; it is of course possible that pH : 6·5 is at or near the limit of tolerance of the species, a point that will be referred to later in the discussion. Wild duck frequented the flats and they may be the cause of only small snails being found in the ditch, but it is difficult to believe that they selectively eat *L. truncatula* however small. In a situation like this it would be a matter of some importance

to know precisely what factors rendered the flats unsatisfactory to this snail : the data which I was able to collect are apparently inadequate to a solution of the problem.

Case 8.

This farm, not far from Rhuddlan, was situated partly on the slopes of a hill of Lower Trias and partly on Alluvial flats extending northwards to the sea. On the slopes were two ponds : one the usual negative farm pond fouled by cattle and ducks, the other further from the farm buildings and higher up the slope. This pond had relatively clean water of *p*H : 7·1 and was never known to dry out even in a rainless summer. Around its margin were some shrubs and rushes where moorhens regularly nested. No snails of any kind were found in either of the ponds.

The flats, carrying the usual sedges and large patches of rushes and normally boggy throughout the year, were intersected by numerous drainage ditches and by a wide, deep " cut " which bore the diverted water of a small river. At the time of the visit the ground was quite dry except at the roots of the larger rushes ; most of the ditches had also dried out. Only one contained a little stagnant water (*p*H : 7·1), and the water in the cut itself was at a low level and barely moving. The flats were known to be flukey.

In the wet ditch and the cut were found numerous young *L. pereger* and several Planorbids, but no trace of *L. truncatula*. The dried marsh and ditches were searched carefully and several empty shells of *L. pereger* were found. In some cases, where the shells lay at the roots of rushes, a living snail was present. Finally, when the search was almost abandoned, a single small shell of (apparently) *L. truncatula* was found by Mr. Rowlands.

This shell is 5·2 mm. long, has the deep suture characteristic of *L. truncatula*, and is extremely frail. It is unusually broad, the ratio of length to breadth being 1·79. I have attempted to show elsewhere (1938) that the ratio : [log (total length) / log (length of body whorl)] is of some value as a species-constant, particularly in differentiating *L. truncatula* from *L. palustris*, to which species this particular shell might conceivably belong. The value of the ratio for this shell, viz., 1·17, is close to the mean for *L. truncatula* and outside the range of variation for *L. palustris*. It is difficult to decide from a single untypical shell, but I am convinced that it is *L. truncatula*.

On the reasonable assumption that aquatic birds do not discriminate between *L. truncatula* and *L. pereger* of comparable size, it is again difficult to account for the relative absence of *L. truncatula* in this admittedly flukey spot. And with such an elusive intermediary it becomes increasingly difficult to understand how *Fasciola hepatica* manages to complete its life cycle at all. One is led to entertain the possibility that certain local races of *L. pereger* may act as efficient intermediaries, in spite of recent German testimony to the contrary.

Case 9.

A visit was paid to the Madryn Farm School at Bodfean (Caernarvonshire), as I was anxious to know something of fluke conditions in the Lleyn peninsula where sheep are numerous in the low-lying rich meadowlands. In the absence on vacation of Principal Isaac Jones, I was received by a member of his Staff who kindly gave me several addresses of fluke-infested farms, mostly in the valley of the Soch, a river running throughout its brief course over Ordovician measures.

One such farm had pastures on low rolling hills and level meadows along the banks of the Soch. On the hill a field with sheep grazing was bordered on all four sides by a ditch that, at this time, was completely dry. A pond in one corner had very little water in it. No aquatic snails were found in this field. A pond, in a riverside meadow where cattle were grazing and geese foraging, contained *L. pereger* and some Planorbis. In the river itself water-weeds along the margins were swarming with young stages of *L. pereger*. Small shallow creeks running in from the river were searched ; *L. pereger* was scanty here but in one such creek I found two small specimens of *L. truncatula* (*p*H : 6·9). The larger was only 4·2 mm. long, with a length/breadth ratio of 1·9. Small as it is, the river Soch appears to be too large and swift to make a natural habitat for *L. truncatula*, and it seems likely that the two specimens had been washed down from higher reaches. As this was known to be a flukey farm, a similar origin may be supposed for the numerous snails that must be present in a bad year. There was no possibility of searching further on this occasion since heavy rain, the first for many weeks, began to fall. I noted the fact that geese were not able to keep down the snail population, and this has since been confirmed (see Discussion).

In travelling across Wales several of the roadside ditches and ponds were examined, but without finding *L. truncatula* ; doubtless many of

these habitats would be positive in a wet season. The few mountain streams examined, notably the river above Llyn Peris, seemed inimical to most forms of life, possibly owing to a common reddish iron deposit.

C. WESTERN ENGLAND.

My indebtedness to Officers in the Western Province is extensive. Early in the course of the investigation the Veterinary Advisory Officer, Mr. D. W. Menzies, M.R.C.V.S., kindly sent me a long list of addresses and has since discussed the problem with me. The list was lengthened through the kindness of Mr. C. Comely, Assistant Agricultural Organizer for Gloucester, and of Mr. Price, Agricultural Organizer for Wilts. The latter interestingly described the close relation between the geology and animal husbandry of that county ; the majority of sheep are kept on the dry chalk downs, with cattle in the wet valleys. Dr. Dawe of the Agricultural Economics Dept. of Bristol University generously lent me his data on sheep distribution in the western province. Dr. C. L. Walton of the Long Ashton Research Station, whose pioneer work in Wales on the bionomics of *L. truncatula* is widely known, very kindly took me to see an unusual habitat on a farm in Somerset and referred me to another on the borders of Worcestershire ; I am obliged to him for many courtesies. It will be convenient to deal with Dr. Walton's cases first.

Case 10.

This is a gated common, on the main road from Tewkesbury to Worcester about two miles from Tewkesbury, on which some of the neighbouring farmers enjoy grazing rights. The broadly oval common is situated on the western edge of the band of Lias which runs diagonally across England. A small stream running beside a farm road enters the common on the east side, turns south and, following the edge of the common, passes under the main road and leaves the common at its southernmost point, to empty into the Severn a short distance away. Towards the northern edge of the common is a duck pond in which no snails were found. *L. truncatula* was found in fair numbers in the ditch alongside the eastern farm road, just outside the common gate, both in the water (pH : 7·6) and out of it on damp mud. Immediately inside the gate is a swampy patch with sedges, and here the snail was found scantily on damp mud at the sedge roots. The day was warm and cloudless but snails out of water were protected by dense vegetation in the ditch and by the sedges on the common.

At that time (May, 1937) fluke had not been serious on the common for two years, when there were heavy losses both here and in the surrounding district. The common has not been seen under wet conditions, but the central and northern part stands high, with a good slope for drainage towards the southeast, and there does seem to be here a case for fencing off the stream and swampy part, and watering stock at the duck-pond.

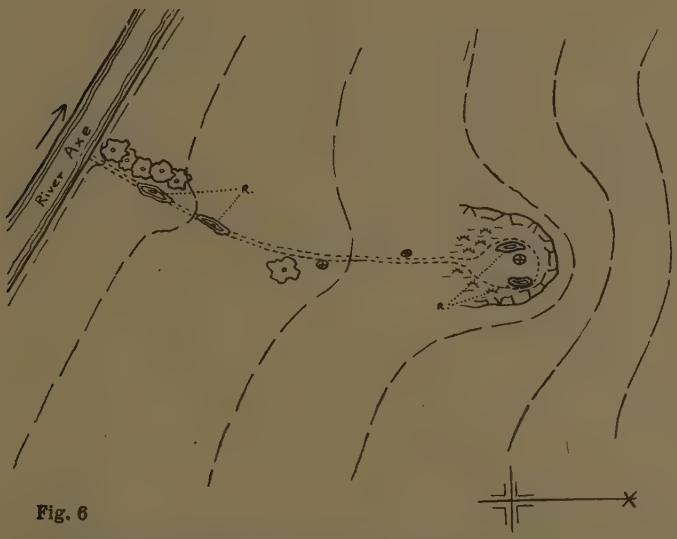

Fig. 6.—Diagrammatic plan to illustrate Case 11; the broken line shows the course of the stream when running; R, residual puddles as at 16/7/37.

Case 11.

This farm, near Axbridge at the foot of the Mendip scarp, was of particular interest in that Dr. Walton had found there *L. truncatula* in a spring pool which regularly dries out every summer (Fig. 6). The pool lies in a slight rocky depression in a rich meadow sloping gently south, and the small effluent stream runs for only about 100 yards when it empties into the river Axe. The meadow is on Carboniferous limestone with Dolomitic Conglomerate underlying, but close at hand the older rocks are unconformably overlaid by the Alluvial plain of Somerset. It is mainly a cattle country; no sheep are bred locally but there is some fattening of lambs.

When the farm was first visited (21/5/37) the spring was still flowing, and *L. truncatula* and *L. palustris* were found together in considerable numbers both in the pool itself (*p*H : 7·3) and in the stream. I had expected the spring water to be very cold but actually (13°C.) it was half a degree warmer than the surrounding air ; it is true that this was relatively early in the day (9 a.m.) and that the previous day had been sunny, but the pool is quite small and would not long retain its heat.

A fortnight later Dr. Walton wrote me that the spring had ceased to flow, but that snails were present on damp mud and there was still water in the pool. I was not able to visit the farm again until the 16th July when most of the pool bed was quite dry. A few residual puddles (*p*H : 7·6) contained no snails ; these, however, were found scantily on damp mud under protecting vegetation. The bed of the stream for some yards was dry, but snails were found on the damp under-surface of stones : 33 were collected in this way of which 11 were living, the remainder being empty shells. Further down, below the point where the stream slightly changes its direction (see Fig. 6), there were more residual puddles (*p*H : 8·1) but no snails. Incidentally a rustling in the grass around the pool betrayed the presence of a foot-long eel, which was replaced in the river whence it had presumably crawled overland.

The river Axe is steep-sided at this point and appears to be several feet deep. On the last visit the river water was some 18 inches below the level of the stream bed so that the stream, if it had been running, would have ended in a small waterfall. I have been unable to find any *Limnaea* along the banks of the river, which is in any case a most unlikely habitat. There can be little doubt that the colony of *Limnaea* species in the pool is indigenous, some of its members being able to survive the period of three or four months each year when the spring is dry. The association of *L. truncatula* with *L. palustris* in the same habitat must be very rare ; at all events this is the only case I have so far come across.

Case 12.

One of the farms listed by the Veterinary Advisory Officer lay in the Vale of Taunton Deane in Somerset, between Taunton and Langport. The land slopes down to the northwest in gently undulating hills of rich reddish soil characteristic of the Upper Trias, the soil on this farm being mostly a medium loam which bakes very hard in dry weather. The stock

was mixed : cattle, sheep, and pigs ; there was some sheep breeding, and some fattening of lambs from the Dorchester Fair, whence Somerset derives most of its lambs.

A seven-acre field at the lowest part of the farm was flat, lying wet in winter, and was crossed by shallow drainage ditches which were dry at the time of the visit. The herbage here was good, however, with very few sedges. *L. truncatula* was found very scantily in this and the adjoining field, in damp spots in the ditches. Much higher up the slope was a flat field which lies wet all the year round, never being dry enough for mowing. Here were numerous tussocks of sedges and rushes with water about two inches deep between the tussocks, and this at a time when the rest of the farm land was too dry to be workable. A sturdy race of *L. truncatula* was found plentifully here, in the water (pH : 7·4) and out of it on damp mud and also on mats of filamentous algae floating on the water. The shells were large (6·2 to 10·3 mm.), slender (length/breadth =2·06), and firm. Being flat, the field did not lend itself to draining, and was regarded more or less as a liability apart from a little cattle grazing. It obviously constituted an ideal permanent habitat for *L. truncatula* which, in wet seasons, would be able to spread along the ditches into neighbouring fields.

Case 13.

This and the following two farms bordering the Severn between Severn Bridge and Gloucester, one on each side and both lying on the Lias, were brought to my notice by the Assistant Agricultural Organizer for Gloucestershire. The first (Fig. 7) is on the north bank. The farm buildings are on a slight elevation from which the land slopes away south-west to a small stream, and south-east to flats bordering the Severn. Landward of a narrow beach is a low sea wall designed to withstand the encroachments of the Severn at Spring tides or in storm. In spite of this the river manages to get over at least once during each year, flooding the flats with what is practically sea water. On the slopes running down to the flats conditions were dry and no aquatic snails were found or expected ; on the flats themselves were several wet ditches, but these also were quite devoid of snails. *L. truncatula* was found in the stream on the other side of the farm, and also along the damp lower border of the neighbouring field. At one point the stream becomes very

shallow where a farm road fords it (see Fig. 7) and here the snails were especially numerous. The reaction of the stream water was *p*H : 7·6.

The interest of this farm lies in the apparent absence of *L. truncatula* from fields subjected to periodical flooding with salt water. I had no equipment for estimating chlorides in the ditches on the flats, but they would almost certainly tend to accumulate there. Evidence of a somewhat

Fig. 7.—Diagrammatic plan to illustrate Case 13.

doubtful character from Germany suggests that sea water is inimical to *L. truncatula* ; the point will be reconsidered in the discussion below.

Case 14.

This farm (Fig. 8) has extensive, flat grazing meadows, known to be flukey, along the southern bank of the Severn and almost exactly opposite the previously mentioned farm. The Severn at this point is over a mile wide at high tide, but I was told that stock had crossed at low tide, on

one occasion at least, from the opposite farm. In the absence of the farmer, I was taken round the meadows by the shepherd. The ground was hard enough for us to drive everywhere in the car, a condition unknown in the previous experience of the shepherd for this time of year (April, 1938). The meadows are intersected by deep reens, which were well filled with water containing numerous water weeds on which *L. pereger* were climbing in profusion. Running diagonally across the largest meadow (of 100 acres) there were shallow ditches, locally called drains, which

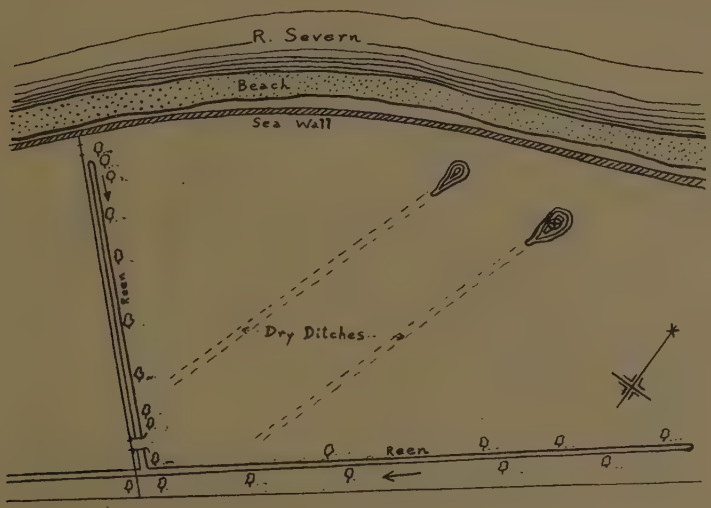

Fig. 8

Fig. 8.—Diagrammatic plan to illustrate Case 14.

end blindly at their lowest point, within the compass of the stout sea wall. These drains have no connection with the reens and appear to lose their water solely by evaporation, unless some seepage occurs through the sea wall. At this time the drains were empty and dry apart from a shallow residual pool at the distal end of two of them (Fig. 8). In one of these pools (*p*H : 8·2) *L. truncatula* was fairly numerous, crawling under an inch or two of sun-warmed water on a substrate of rather soft clay. In the adjoining pool, a few yards away, there were no snails but hundreds of small caddis fly larvae. The reaction of this pool was outside

the range of the comparator discs (*p*H : 4·8–8·8) but the B.D.H. "Universal" indicator gave a colour which I judged to be equivalent to *p*H : 9·4. The shepherd said that it was extremely rare for a tide to get over the sea wall but, in view of the possibility of seepage referred to above, a chloride estimation might have been instructive.

These flats are the haunt of wild ducks and geese, shooting rights being let, so that the question arises whether wild ducks are as efficacious as their domestic congeners in eating *L. truncatula*. It is true that the snails were found only in one small residual pool, but they were quite numerous and in no way hidden from view. And it is evident that in less droughty seasons they must be widespread; the dangerous nature of the meadows is reflected in the management system, for ewes are bought in for lamb-fattening only, and are sold again within the year.

Apart from the drought situation on this farm, there is some interest in the high *p*H-values of residual puddles, and the absence of the snail from a pool of *p*H : 9+; this point will be referred to in the discussion.

Case 15.

I am indebted to Mr. Menzies for an introduction to this case which has interested the local authorities since it involves a disease in dairy cattle ascribed to fluke. The farm lies in Wilts. between Malmesbury and Wootton Bassett, on rich land sloping south towards the South Wales line of the G.W.R. The district, which is on Oxford Clay (Middle Oolite), is predominantly given over to dairy farming and this particular farm was unusual in carrying a flock of some 200 sheep in addition to about 90 head of cattle. Four springs in as many fields adjoining the railway normally give rise to narrow strips of wet land running down the slope, the water being carried under the railway embankment to enter, a short distance away, a tributary of the Bristol Avon. The first spring was fenced off from cattle apart from a small drinking basin and, in spite of continuing drought, the spring was still active. The spring pool was overgrown with a coarse lush grass and there were traces in the water of a reddish iron deposit. Two small specimens of *L. truncatula* were found here (*p*H : 6·9), but no other snails. The second and third springs were almost dry, being represented by boggy patches with no effluent; a small hollow whence the third spring rises contained clear water giving a reaction of *p*H : 7·1, and a single specimen of *L. truncatula*

was found close by on damp mud. Another was found at the fourth spring which feeds a disused pond. No empty shells were found, and the four living specimens were the sole fruits of a two-hours' search. No snails were found in a ditch running along the foot of the railway embankment. Proposals were under consideration for treating the fourth spring with copper sulphate, draining the second and third underground, and piping the first as a farm supply. It was difficult to believe that fluke had been serious here in recent years, unless it was already latent in cattle bought in, but in this respect the farm is comparable with many of those in Wales.

D. SOUTH DEVON.

I am indebted to the Veterinary Advisory Officer of the South-western Province, Mr. C. V. Watkins, M.R.C.V.S., D.V.S.M., for the address of a veterinary surgeon practising in a flukey area in north Cornwall. Unfortunately, I have received no reply to a letter sent to him.

From Mr. R. S. Roberts I had obtained the address of Mr. J. H. Fairweather of Kingsbridge, who is the secretary of a farmers' club in the locality. To him I am indebted for a number of introductions to farmers, having been able to meet him and them at the sale of South Devon bulls held at South Brent in April of this year. This southernmost section of the county is quite characteristic ; low rolling hills of red soil derived from the Devonian measures drop steeply down into river valleys which, near the coast, are often drowned by the sea at high tide, like the drowned valleys of south Cornwall. The rich herbage supports the South Devon breeds of cattle and sheep, both of which originate from this area. Fluke is intermittently reported from farms lying within the triangle : Totnes—Plymouth—Salcombe, and is a serious menace on some farms. Two will be selected for comment.

Case 16.

This farm, bordering a salt-water estuary, gave a history of fluke in sheep during the previous winter and spring. The farm buildings were situated on a hill (Fig. 9) with very steep slopes running down abruptly to the estuary, One field to the south-west, regarded as flukey, was quite dry apart from a small spring feeding a silted-up pond at the very bottom. *L. truncatula* was found very scantily in the spring effluent (pH : 7·9). Another field to the south had springs half way up the slope and streams running down the hedgerow ditches to operate a water-ram on their way to the sea ;

water weeds were abundant in the ditches and on these *L. pereger* was found, but no *L. truncatula*. In a third field to the south-east, high up the slope, was a bay enclosing a boggy patch fed by a spring and trampled by cattle, and here again *L. truncatula* was found scantily. In the same field was a raised drinking trough with a piped water supply ; this was free from snails. In both of the infested fields it would have been a relatively simple matter to fence off the dangerous areas, as the farmer readily agreed to do ; instead of which, acting under advice, he had been

Fig. 9

Fig. 9.—Diagrammatic plan to illustrate Case 16 ; S, spring.

laboriously liming the steep slope of the first field (where no *L. truncatula* would be likely to gain a footing even in the wettest season), and had hacked a small groove out of the hard dry ground around the spring and filled the groove with powdered copper sulphate at no small expense. A tenth of the quantity of copper sulphate made into a solution and sprayed on the wet mud of the pool might have done some good. The farmer was under the erroneous impression that *L. truncatula* can travel long distances over dry ground.

Case 17.

This farm, near Modbury, was of a similar conformation to the last, but further from the sea. The flukey fields sloped steeply down to a stream (*p*H : 7·8), but patches of rushes and iris high on the slopes betrayed the presence of springs which gave rise to strips of waterlogged land right down to the stream. There had been no losses from fluke on this farm since 1933, and sheep were only rarely turned into these wet meadows, in summer. Conditions appeared well suited to *L. truncatula* apart from the presence of a large flock of ducks with a free range over the wet fields, and to them must be ascribed the failure to find a single snail of any kind.

E. DORSET.

Through the good offices of Mr. N. S. Barron, B.Sc., M.R.C.V.S., Veterinary Advisory Officer of the Southern Province centred at Reading, I was put into touch with Mr. R. Wightman, Assistant Agricultural Organizer for Dorset, who has been most helpful. He explained that in Dorset sheep are mostly on arable farms or on the chalk downs, and are therefore not subject to fluke, but the disease has become important in cattle in recent years, especially in Marshwood Vale, near Bridport, and in Blackmoor Vale. The latter is a region where agriculture is thought of in terms of gallons of milk per acre, and sheep are seldom seen.

Case 18.

I had the privilege of being taken to see a farm in the Blackmoor Vale by the Director of Agriculture, Mr. Ferris, accompanied by Mr. Wightman; I am indebted to both for the trouble they took. The Blackmoor Vale lies on Oxford Clay (Middle Oolite) and is hemmed in on all sides by hills apart from a narrow valley in the south, which carries the river Stour out of the Vale to Blandford. The farm in question had several flat, low-lying meadows which are wet throughout most of the year and often too wet for mowing. There had been repeated losses from fluke in cattle (no sheep were kept) in recent years, and these marshy meadows were known to support a large population of *L. truncatula*. So severe had been this year's drought, however, that the meadows were quite dry (April, 1938), and so were most of the drainage ditches. A stagnant pond in the centre of one field (*p*H : 8·1) yielded no snails. A few residual puddles in the drainage ditches were foul, and without snails. Careful search by the

three of us yielded no aquatic snails of any kind, not even empty shells, though these were confidently expected among the grass roots in the depressions. Nearby roadside ditches, mostly quite dry, gave similar results. The Director of Agriculture had never seen these meadows so dry, even in August. I am very glad to have seen this farm where *L. truncatula* has been known to occur in large numbers, and glad to have had expert assistance in looking for the elusive snail there, because in many of the other negative cases the evidence for the past occurrence of *L. truncatula* was indirect, and the evidence for its present non-occurrence was unsupported. As in so many of the cases, the really baffling feature was the complete absence of even empty shells or fragments.

Case 19.

Mr. Wightman kindly took me to see a water meadow near Dorchester, situated on Eocene measures. Water meadows are a feature of the south coast, in flat river valleys where the river can be dammed so as to give an appreciable difference of level. The general scheme of such a meadow is illustrated diagrammatically in Fig. 10, in plan and section. The whole meadow is divided into a series of ridges and furrows, several yards apart. Feeding channels, which can be connected at will with the river above the dam, are cut along the crests of the ridges, while drainage channels cut along the furrows lead the water back to the river below the dam. Meadows are "flooded" on some date in December, depending on climatic and other conditions, and the supply is cut off towards the end of March. In spite of the term "flooding," there is no standing flood but a trickle of water down the slopes from ridge to furrow. The effect is a tremendous growth of lush herbage on which sheep are grazed (in this locality) from early April for about six weeks, and cattle throughout summer and autumn. A local tradition has it that if sheep are left on into June they will die within three months, and this may possibly refer to fluke. After the water is shut off the channels should dry out if they have been properly made and attended to, but in one meadow visited there was still some water in the furrow channels (29th April) and here *L. truncatula* was found in plenty. It was absent from the river and from the main drainage channel. The river gave a reaction of *p*H : 8·1 and the main drain *p*H : 7·9, the water in the latter being of course stagnant. In this meadow the channels were about 12 inches wide and 9 inches deep. In another meadow, where the channels were smaller and better cared for,

they were dry and no snails were found. There can be no doubt that, where water meadows are not maintained in perfect order, allowing some water to remain in the channels throughout the summer, conditions are ideal for the breeding of *L. truncatula*.

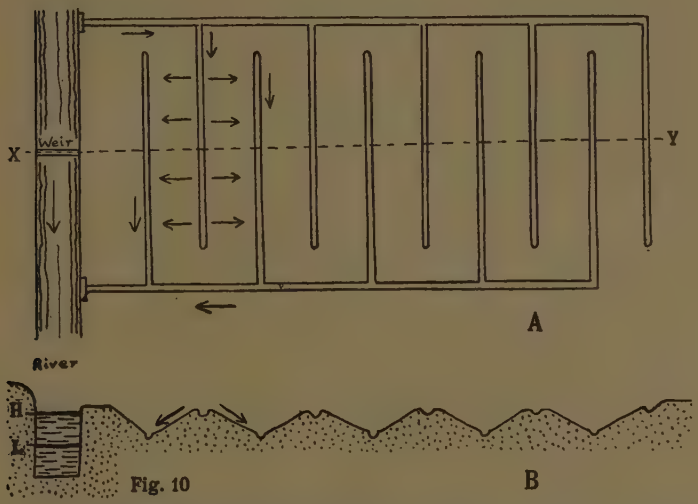

Fig. 10.—Simplified scheme of a water meadow to illustrate Case 19; A, plan; B, section X–Y; H, High water level above weir, L, low water level below weir. The slopes are exaggerated.

F. THE COLNE BASIN.

The main tributaries of the Hertfordshire Colne drain the eastern slopes of the Chiltern Hills and are more or less parallel, running from the north-west towards the south-east. The Colne itself is roughly at right-angles to its tributaries, running south-west to Rickmansworth and then due south, to empty into the Thames at Staines. These western tributaries and the Colne itself for most of its length are all on Chalk overlaid by Glacial boulder clay, but the head-waters of the Colne and a small eastern tributary draining the Elstree-Radlett region, the Tyke's Water, are on the London Clay. The area is not noted for fluke but has some likely breeding places for *L. truncatula*, and its proximity to the

Institute has led to its being much sampled. Unfortunately, *L. truncatula* is very scanty in the area, and not only in dry seasons. The experience of the late Prof. A. E. Boycott, who made detailed observations of molluscs for many years—particularly in the parish of Aldenham—bears this out. In a letter he wrote : " My impression is that *truncatula* is quite uncommon in the Colne valley [*i.e.*, excluding tributaries]. On the London Clay between Radlett and Elstree . . . it is widely scattered in ditches, plashy places, disappearing ponds, etc., but here again I never saw more than odd examples except in one place at Boreham Wood, where it was abundant for many years." I have visited this field five times in six months and, although *L. truncatula* was found very scantily at the first visit, it has not been seen since ; *L. peregeri* has always been present in small numbers. The reaction of the water has varied from *p*H : 7·2 to 8·1.

The numerous negative habitats of this area are of no special interest since collecting has been largely at random and not by way of flukey farms. But there are features that call for mention in one of the few positive habitats, one brought to my notice by Prof. Leiper who had found *L. truncatula* there.

Case 20.

This is a farm road and bridle path, passing by a bridge under the L.M.S. Railway near Radlett, and on the London Clay. On one side, adjoining the brick wall which forms the arch of the bridge, a small sluggish stream is carried in a channel about a foot wide. The stream appears to emerge from the ground at one end of the bridge and to disappear again at the other, where it probably percolates underground to the river a few yards away. A water-weed, probably *Apium nodiflorum*, grows luxuriantly in the channel. The roadway under the bridge is floored with stone sets which have become covered with a thin layer of mud, and is slightly flooded after heavy rain. The water in the channel has shown a progressive slight increase in alkalinity during the period from May, 1937 (*p*H : 7·3) to March, 1938 (*p*H : 7·8).

On the first visit (4/5/37) *L. truncatula* was found in fair numbers in the water of the channel and on the damp mud alongside, but no other snail was present. On the next visit (20/7/37) *L. truncatula* could not be found but *Hydrobia jenkinsi* was numerous in the water, which this species does not leave, being an operculate. On the next visit (24/9/37)

the *H. jenkinsi* were very scanty and one young *L. truncatula* was found. Next (25/2/38) both species were scanty, *L. truncatula* being confined to the mud. On the last visit made (31/3/38) it was difficult to find either of them; after a long search 2 *L. truncatula* and 5 *H. jenkinsi* were recovered. February and March had given an excessively low rainfall in this district—the Institute records show 0·61 inch and 0·39 inch respectively, compared with a monthly mean of about 3 inches—and this may have been an adverse factor. On the other hand the habitat is a small one and is apparently isolated except possibly in time of flood, so that the mere collection of snails would probably have a major effect. The interesting feature is the arrival of *H. jenkinsi*, for the first time at this place so far as I can discover, in a habitat that does not seem ideally suited to it. According to Boycott (1936), the disappearance of other species of mollusc when *H. jenkinsi* overruns a locality has been noted by Stelfox, Welch and others; this might be a suggestive line of enquiry but for the fact that *L. truncatula* and *H. jenkinsi* rarely occupy the same habitat.

G. MISCELLANEOUS.

Case 21.

A. P. Thomas collected the material for his life-history studies in the neighbourhood of Oxford, and in one place he explicitly refers to the village of Wytham. In the September of 1936, I was privileged to accompany Prof. Leiper on a collecting tour of the Oxford area. Around Oxford it was impossible to find *L. truncatula* except at one place, on the Oxford Clay near Wytham, where a stream runs down a sloping field from the low hill behind, is piped under the road, and trickles through a flat meadow to reach one arm of the Thames a few yards from the road. In the meadow, where bullocks were grazing, the stream makes a boggy patch much trodden by cattle, and *L. truncatula* was found in considerable numbers in flooded hoof-prints and on damp mud nearby. I have often searched hoof-prints around farm ponds in vain: the water is stagnant and foul, and quite unsuited to the snail. But here where the hoof-prints were washed by a clear stream the conditions seemed ideal. On two subsequent visits (28/7/37 and 3/2/38) the snails have been present, but in small numbers.

In the quotation cited above (p. 214) Thomas refers to "Breeding haunts in marshy places up the river." I have not yet worked over the

headwaters of the Thames with any thoroughness, but preliminary searches in the river Coln near Coln St. Aldwyn and Whelford, and in the very numerous roadside ditches around Minety (April, 1938) failed to reveal any specimens. Most of these ditches, like the reens near Cardiff (Case 1) are too deep to be satisfactory habitats.

Case 22.

Roadside ditches may be important where the flow is reliable, the water not very deep, and the current not too strong. Those ditches which merely drain the road are not important habitats. A case of the former kind is a ditch on the west side of the main road from Wantage to Oxford, about one mile north of Wantage. The country here is low-lying, approaching the flats of the Thames Valley, and the geological measure is Upper Greensand.

When first visited (3/2/38) the ditch was running, had a gravelly bottom with small patches of peaty mud, and contained many decaying leaves. *L. truncatula* and *L. pereger* were both plentiful ; the size of the snails (*L. truncatula* averaged about 7 mm. long) made it certain that they were survivors from the previous season. These two species were again numerous at the next visit (15/3/38) when several planorbids and a single *Aplecta hypnorum* were also found. The snails were all under water (*p*H : 8·2) about three inches deep and were mostly crawling over the decaying leaves upon which they appear to feed. Two months later (9/5/38) the ditch was without water, owing to the absence of normal rainfall for over three months. No snails could be seen on the undisturbed bottom, but they were found plentifully under layers of drying filamentous algae. The algal mat, which must have formed on the surface after the ditch had ceased to flow, had gradually subsided with the decreasing water, keeping the snails below it as in a net. Although the upper surface of the mat was dry the layers below were still moist, with the result that the snails remained alive. Of 85 *L. truncatula* collected, 80 were living. 46 *L. pereger* and 23 *A. hypnorum*, all living, were also collected on this occasion. Filamentous algae and *L. truncatula* are not often associated, but the importance of the former in a case like the present is obvious ; they first appear when the stream stagnates and then form a protective blanket when it finally dries out.

Discussion.

The 22 selected case-reports in the preceding section afford a basis for a brief and preliminary discussion on some of the factors which control the suitability of habitats for *L. truncatula*. It will be convenient to deal with each factor under a separate subheading.

ALTITUDE.

The undoubted fact that *L. truncatula* favours wet spots has led to the popular *non sequitur* that it is found only at very low altitudes. None of the farms visited so far has been at a very high elevation, but Walton (1917, p. 244) found the snail in the Aberystwyth area at altitudes of 1,250 feet, "And there is no doubt that they would range higher were conditions right." Boycott (1936, p. 147) quotes references to *L. pereger* at 2,400, 2,000, 1,750, and 1,600 feet, and *L. stagnalis* at 1,250 feet, but he does not deal with *L. truncatula* in this paper. One comment of his illuminates Walton's reference to right conditions: "Since practically all the ground [in this country] over 1,500 feet is on the older, siliceous rocks, the water in high loci also differs from that in lower places by being soft and often very soft." Altitude is likely to limit *L. truncatula*, then, not through temperature (see below) but through available calcium for shell building. Further, on the small scale of a single farm, it is not always the low-lying land which is infested: this is shown by Cases 2, 7 and 12 where *L. truncatula* was scanty or quite absent in the flats but plentiful at higher altitudes. Cases 6 and 16 show that marshy spots can occur high up on a steep slope.

GEOLOGICAL FORMATION.

On the Continent there is a tendency to regard *L. truncatula* as conditioned to some extent by the geological features of the locality; thus, Mehl frequently refers to it as occurring on the Keuper (Upper Trias) in Franconia. Geological features of the 22 cases are briefly summarized in the accompanying Table, which shows that the snail has been found on Alluvium, Eocene, Upper Greensand, Middle Oolite, Lias, Upper Trias, Carboniferous Limestone, Devonian, and Ordovician measures. Boycott's point that the older rocks may yield waters too soft for snails has already been made. At the other extreme, Chalk is unsuitable if only because of its porous nature, and fluke is practically unheard of on our chalk downs. But apart from these extremes, it appears that any geological measure is satisfactory which will supply a stiff

retentive soil on which perennial streams can establish themselves. Clays of various kinds are represented in all the major geological divisions from the Trias upwards, except the Chalk, and glacial drifts of Boulder-clay widely cover rocks of all divisions except in the south of England.

SUBSTRATE.

Apart from its value in retaining bodies of water, clay appears to be a favourite substrate for *L. truncatula* to crawl over. Silty muds are also satisfactory if they are not too soft and flocculent, in which case *L. truncatula* sinks in and is lost. Walton (1917) found that *L. pereger* with its relatively broader foot could crawl with safety over muds into which *L. truncatula* would sink, and my observations bear that out. For the same reason peat is unsatisfactory; Walton considers that deficiency in calcium may be a contributory factor in this case. I have never found *L. truncatula* on coarse sand, and only rarely on stones.

HYDROGRAPHY.

This term is used here to cover the general nature of the fresh-water environments in which *L. truncatula* is found. In a survey of the fresh-water molluscs of Aldenham, Boycott (1919) usefully distinguishes habitats as river, lake, stream, running pond, and closed pond; for present purposes marsh and drainage ditch must be added, and the lot may conveniently be symbolized by initial letters as follows: R, L. S. P, C, M, D, which are used in the Table. It will be noted that 12 of the habitats are streams of which the essential features are small cross-section and running water (I have included springs under the same heading); three are drainage ditches (the roadside "ditch" of Case 22 was not merely a ditch to drain the road, but was a running stream); two are marshes in the sense of waterlogged fields; one is a running pond, and one is a river. Of the latter it may be said that the Soch is a "river" largely by courtesy—some would call it a stream—and in any case the two specimens found there were probably not in a natural habitat. The principal requirements seem to be that the water should be shallow, and that it should be running or at least should run at some season of the year. Even the boggy fields of Cases 2 and 12 must have been fed with springs in order to maintain wet conditions through a drought, so that in a sense they were running. Closed ponds are not represented, though I have examined several; the average farmpond is too foul and/or duck-laden for snails. Woodland ponds are usually

lacking in most forms of animal life, owing to the continuous deposition and decomposition of trash—leaves and twigs from the trees.

TABLE.

Presence of *L. truncatula*, geology, hydrogen ion concentration, and type of habitat in the 22 described localities. (For explanation of habitat symbols see text, p. 250.)

Case.	*L. truncatula*.	Geological Formation.	*p*H.	Habitat.
1	+	Alluvium	—	S.
2	+	Lower Lias	7·3	M.
3	—	Ordovician	7·2	—
4	—	Devonian	7·0	—
5	+	Devonian	7·3	S, P.
6	+	Devonian	—	S.
7	—	Ordovician	6·5	—
	+	Ordovician	6·9	D.
8	—	Lower Trias	—	—
	—	Alluvium	7·1	—
9	+	Ordovician	6·9	R.
10	+	Lias	7·6	S.
11	+	Carboniferous... ...	7·3	S.
	—	Carboniferous... ...	8·1	—
12	+	Upper Trias ...	7·4	M, D.
13	+	Lias	7·6	S.
14	+	Lias	8·2	D.
	—	Lias	9·4	—
15	+	Middle Oolite ...	6·9–7·1	S.
16	+	Devonian	7·9	S.
17	—	Devonian	7·8	—
18	—	Middle Oolite ...	8·1	—
19	+	Eocene	7·9	S.
20	+	Lower Eocene ...	7·3–7·8	S.
21	+	Middle Oolite ...	—	S.
22	+	Upper Greensand ...	8·2	S.

TEMPERATURE.

My ranges of water temperatures are narrow and therefore not very informative. Boycott (1936, p. 168) points out that in shallow loci the water temperatures are quickly controlled by air temperatures and sunshine : the latter may carry the water temperature much higher than that of the adjoining air, especially at shallow margins and where much vegetation impedes mixture. High temperatures are not likely to be directly inimical at these latitudes, but their indirect effect of hastening putrefaction in some loci may act adversely by using up the available supply of oxygen which in turn is less concentrated at higher temperatures.

Mozley (1937) shows that *L. truncatula* is found in the sub-arctic fauna, and Mehl (1932, p. 77) points out that it is unharmed in loci which do not freeze to the bottom, and can even withstand being frozen solid in mud at −8°C. for several days. Temperature is therefore not likely to be an important controlling factor in itself.

MINERAL CONSTITUENTS OF WATER.

Calcium in small quantities must be essential for building the shell, and where it is seriously deficient the shell is thin and friable (Walton, 1917, p. 245; Peters, 1938, p. 184). That this is not an overriding consideration, however, is surely shown by the fact that fluke is commonest in the least calcareous parts of England and Wales.

Case 13 suggests that chlorides, in concentrations of the order of sea water, are inimical, but the evidence is not very strong. The literature on this point is conflicting. In a series of papers Lührs (1933A), dealing with the control of fluke in lands bordering the sea (Oldenburg), claims that flooding ditches with sea water is efficacious. In another periodical, however, Lührs' experiments are attacked by Mehl (Lührs, 1933B; Mehl, 1933; Mehl & Lührs, 1934) on the ground that Lührs had not even found *L. truncatula* in his locality! Walton (1922) reports that dressings of salt heavy enough to injure the grass destroyed no more than 70–80% of the snails.

The note appended to Case 9 suggests that reddish iron deposits may be inimical, but this is not borne out by Case 15. As with so many of these physico-chemical factors, there is a pressing need for laboratory investigation to supplement field observations.

HYDROGEN ION CONCENTRATION.

The Table shows that *L. truncatula* has been found living in waters of *p*H: 6·9 to 8·2. It was not found in water of *p*H: 6·5 (Case 7) or of *p*H: 9·4 (Case 14), although in both cases it was living close at hand; but this merely *suggests* toleration limits—the decisive factor in both cases may well have been other than *p*H. Atkins & Lebour (1924) found the range of tolerance from 16 samples to be *p*H: 6·4 to 7·8. Walton & Wright (1926) increased this range, for 26 positive samples, to *p*H: 6·0 to 8·6, and this extends beyond my range at both ends. They had only one sample higher (*p*H: 8·8) and three lower (*p*H: 5·6 to 5·8), however, so the actual range of tolerance may be greater; their most frequent value was *p*H: 7·4.

Mehl (1932, p. 25) publishes a series of values, determined twenty-four hours after collection, which fall within Walton & Wright's range. Boycott (1936, p. 161), dealing with fresh-water snails in general, states that " Frankly acid peaty water of *p*H : 6 or less contains no snails," while molluscs are abundant at " The highest figure of about 8·5 found in this country." He points out the difficulty of interpreting results and of deciding whether to take the *p*H of the water as it stands or after bringing it into equilibrium with air, and the impossibility of making a scale relating *p*H to calcium content " Which seems to be the more important factor." A bottle of indicator, he concludes, is a good servant but a bad master.

MIGRATION FROM WATER.

In the majority of the preceding cases *L. truncatula* has been found under water ; in a few cases it was on damp mud close by. Walton (1917, p. 257) states that the snail " Was never found to have crawled voluntarily more than a few inches away from water," and Mehl (1932, p. 83) concludes that under ordinary conditions it remains in the water—" When oxygen is lacking they move a few centimetres away from it, in rare cases as far as one metre to the side." The tradition which regards *L. truncatula* as practically a land snail seems to be based mainly on two considerations : (i) Floods will often spread the snails over a meadow where they are discovered some time after the waters have subsided ; it is then erroneously assumed that they have crawled there. (ii) The snail readily crawls out of water which for some reason (such as oxygen deficiency) is unsatisfactory, and this very frequently happens in aquaria ; the snails crawl only a short distance, however, when they settle down with the mouth of the shell closely applied to the side of the aquarium, and they die of drought rather than return : I have noticed this repeatedly. While there are obviously cases where meadows have been recently flooded and can advantageously be subjected to chemical treatment, there is no point in treating steep dry slopes (Case 16). Much economic waste may be caused by the fallacy that *L. truncatula* makes a habit of invading dry land.

ASSOCIATED ORGANISMS.

There is a great deal of discussion in the literature on this point. Many claim to find definite species of water weed so closely associated with *L. truncatula* as to be indicator-plants, but each claim is subsequently

invalidated by others. So far as other species of snail are concerned, I have found *L. truncatula* associated with *L. palustris* (Case 11), *L. pereger* (Case 9), *Hydrobia jenkinsi* (Case 20), and *L. pereger*, *Aplecta hypnorum*, and planorbids together (Case 22); but usually it is the only mollusc present. Walton (1917, p. 243) found a difference in habitat between *L. truncatula* and *L. pereger*, and Mehl (1932, p. 23) between it and *L. palustris*. Plentiful data and a precise index of association (such as that described by Mozley, 1936) are required for elucidation of this problem.

PREDATORS.

There is no doubt that domestic ducks play an important part in controlling *L. truncatula*. They, with or without moorhens, have been invoked in the present paper to explain the absence of snails in Cases 3, 4, and 17, but without positive evidence, and they were associated with extreme scantiness of snails in Case 5. Case 9 suggested that geese are not eaters of small snails and Mehl (1932, p. 103) points out that the domestic goose is almost exclusively vegetarian, and describes experiments which showed that neither they nor fowls will normally eat the snail. He discusses ducks in detail and suggests that the Indian Runner ♀ × Peking ♂ cross would be a useful breed for prophylactic use: the Indian Runner is a good forager but a small table-bird, whereas the cross is said to have both qualities well developed. Boycott (1919, p. 7) has a footnote on ducks eating aquatic snails in general: "Ducks do not seem to utterly destroy snails but only to so reduce their numbers that they become exceedingly difficult to find. Once the ducks are taken off, . . . snails . . . may quickly reappear."

Conclusions.

The ideal habitat for *L. truncatula* would appear to be a shallow stream or running pond with a low rate of flow so as not to sweep the snails away, floored with clay or fine silt or mud firm enough to support the snail. Being shallow the water will be well aerated, and although it may also be subject to extreme fluctuations in temperature this does not appear to matter. Preferably the water should be permanent but, if not, there should be close vegetation to retard evaporation, to maintain a humid air during periods of drought, and to hide the exposed snails from birds. The water should contain a little calcium and should give an immediate reaction, say, between *p*H: 7·0 and 8·0. Possibly chloride

ions, and certainly copper ions, should not be in excess of the normal for stream waters. Also, the locality should not be frequented by ducks. This is a provisional specification, and further work will no doubt enable more details to be filled in. But the point suggested is that a detailed specification is desirable, and indeed necessary, before new methods of control can be elaborated.

It is an unfortunate fact that a wide interest is taken in liver fluke only at those times when the disease reaches epizootic proportions, that is to say when *L. truncatula* is most numerous, most widespread, and most difficult to control. During the past few years climatic factors have conspired to reduce the numbers of this snail in this country very considerably, so that it occurs plentifully only in a very few favourable habitats; of the cases considered above, the snail was really plentiful only in waterlogged pastures (Cases 2 and 12), in a water meadow (Case 19), and in a roadside stream which normally does not dry out (Case 22). It is a pity that methods of control already tested and found adequate, such as draining, dressing with copper sulphate, or the use of domestic ducks, cannot be widely applied at a time like the present when the snail is in many ways most vulnerable.

Field data of the present type appear to be of some value and are probably worth extending over a period of years, but there is need also for laboratory investigations to elucidate problems touched upon in the discussion, and many others not mentioned. I have attempted to do something in this direction during the past two years, in intervals between sampling, but the chief obstacle has been shortage of material. One must rejoice at the scarcity of *L. truncatula*, but not without a sneaking envy of A. P. Thomas and his 500 snails to a single sweep of a small hand-net!

Addendum: Liver Fluke in England and Wales.

Although the present enquiry has been concerned with *L. truncatula*, the method of approach by way of flukey farms and the valuable information gleaned from Mr. R. S. Roberts, from the Veterinary Advisory Officers and others, and from farmers themselves, have incidentally yielded data on the distribution of fluke in England and Wales. Now, it is very difficult to obtain published data on this matter, and it has therefore appeared desirable to append a provisional map (Fig. 11) together with

these brief comments. This is done with considerable apprehension lest the map be misread. The map is provisional and incomplete. So far as it goes, it can be relied upon as showing centres where fluke does occur or has occurred within recent years. But the boundaries to such centres are necessarily vague and are therefore marked with radial lines to suggest this. Moreover, there are probably many other areas yet to be filled in; in other words, the absence of an indication on the map does *not* imply definite absence of fluke, but merely absence of data.

It is difficult to define "presence of fluke" in a clear and useful way; the phrase may stand for anything from the local butcher's having found an occasional fluke in a liver, to the loss of sheep and cattle from fascioliasis. In the former sense fluke is probably present throughout the country. I have therefore endeavoured to restrict the indications to areas where serious disease, if not death, has occurred.

No useful purpose would be served by distinguishing between sheep and cattle as hosts; the two distributions usually coincide, with a much lower incidence (of recognized cases) in cattle. Where they do not coincide it is usually because one of the hosts is rare or absent (e.g., there are practically no sheep in Blackmoor Vale, Dorset, but fluke occurs in cattle). In hosts there may sometimes be a differential incidence from one season to another; Capt. Williams of Abergavenny told me that in his experience fluke tends to be more marked in cattle after *dry* seasons, when the cattle are driven to eat long rank grass in wet spots—grass which sheep will not touch.

To assist in fixing positions on the map, several towns in the south and west are shown by numbered spots of various sizes, and a key to these is given. The towns and fluke areas are located with considerable precision by means of the coordinates shown in the margins. The units shown are of 100,000 yards, and their subdivisions of 10,000 yards, the origin being a point to the south-west of Great Britain. The 10,000 yard coordinates are engraved as a graticule on the 4th Edition of the Ordnance Survey "Quarter-Inch" maps, the lines being therefore about 1·4 inches apart. It is a simple matter to fix the coordinates of any town or small area on these maps, and thence to determine its position on the outline map.

SUMMARY.

1. The complacent attitude that fluke is no longer a disease of importance is attacked on the grounds that the recent low incidence of the disease is largely due to a temporary scarcity of the intermediary, *Limnaea truncatula*.

2. A description is given of methods of making contacts, taking samples, and recording observations in a survey of typical habitats of *L. truncatula* in England and Wales.

3. Twenty-two such habitats are described in some detail.

4. These form the basis of a brief discussion on factors contributing to the ideal habitat, knowledge of which is desirable for the elaboration of new methods of control.

5. A provisional map showing the distribution of liver fluke in England and Wales is appended.

KEY TO NUMBERED TOWNS ON MAP.

1. Abergavenny
2. Aber-Soch
3. Aberystwyth
4. Axminster
5. Aylesbury
6. Bangor
7. Barnstaple
8. Basingstoke
9. Bath
10. Bedford
11. Birmingham
12. Bodmin
13. Brecon
14. Bridgwater
15. Bridport
16. Bristol
17. Bude
18. Builth Wells
19. Caernarvon
20. Canterbury
21. Cardiff
22. Carmarthen
23. Cheddar
24. Chelmsford
25. Cheltenham
26. Colchester
27. Dolgelley
28. Dorchester
29. Exeter
30. Gloucester
31. Great Torrington
32. Haverfordwest
33. Kingsbridge
34. Leamington
35. Llandilo
36. Llanerchymedd
37. Llangefni
38. Llanwrda
39. Maidstone
40. Modbury
41. Monmouth
42. Moreton in Marsh
43. Newtown
44. Northampton
45. Okehampton
46. Oswestry
47. Oxford
48. Plymouth
49. Reading
50. Rhuddlan
51. St. Albans
52. Salisbury
53. Shaftesbury
54. Shrewsbury
55. Southampton
56. Swansea
57. Swindon
58. Taunton
59. Tewkesbury
60. Torquay
61. Totnes
62. Truro
63. Tunbridge Wells
64. Winchester
65. Wolverhampton
66. Worcester
67. Yeovil

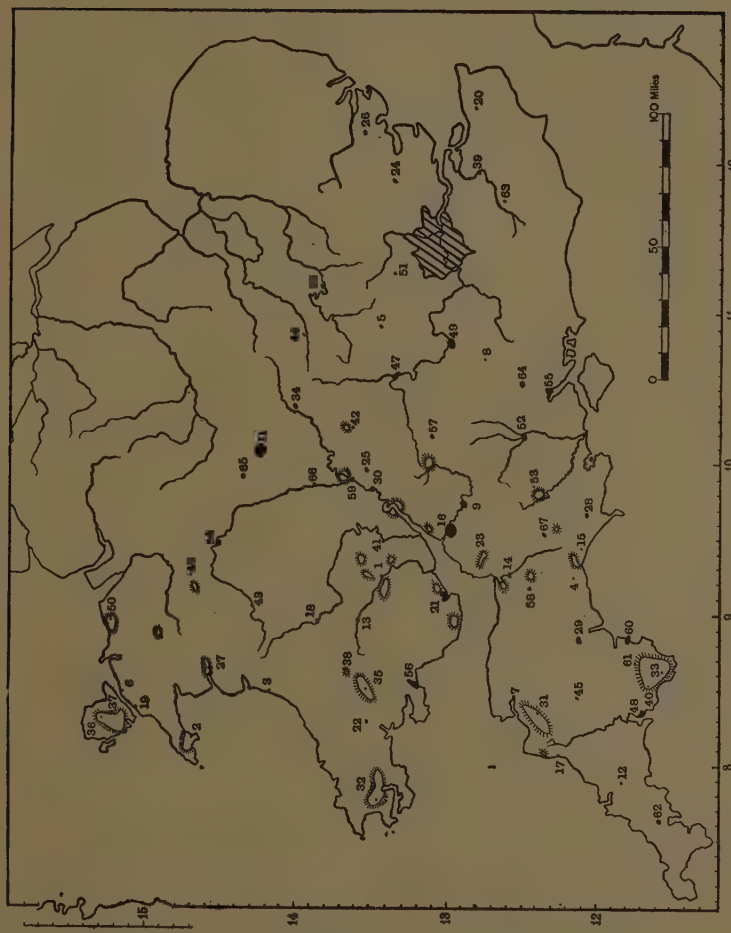

Fig. 11.—Provisional map showing areas (with a ciliated boundary) of recent fascioliasis in Wales and southern England; for towns corresponding to numbered spots, see accompanying key.

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